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C O N T E N T S

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Dr. Walther Rabe:	
Studies on the action of wettable sulphur against conidia of <i>Venturia inaequalis</i> (Cke.) Wint. . . .	1

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BAYER LEVERKUSEN (GERMANY)

Department for Plant Protection

Studies on the action of wettable sulphur against conidia of *Venturia inaequalis* (Cke.) Wint.

by Dr. Walther Rabe, Leverkusen

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A. Introduction and formulation of the problem

The effectiveness of a fungicide is not determined solely by the chemical composition of the active substance. This fundamental fact was established upon comparing the effectiveness of fungicides with an equal content of active substance, which had been prepared in different ways. An interesting example of this is provided by wettable sulphur.

The wettable sulphur preparations sometimes differ greatly in their fungitoxic properties, even when they have the same sulphur content. One of chief causes for this difference in effectiveness is to be found in the type of sulphur used as the starting product (Feichtmeir, 1949 and 1952) and in the process used for preparing the product (Foreman, 1910; Young, 1925; Smith, 1930 b; Frear, 1948, P. 231/232). Dependent upon the method in which it is produced, the starting product has differently shaped particles of varying sizes (Tisdale, 1925; Sauchelli, 1933; Groves, 1942). The suspensibility and adhesiveness of sulphur may be influenced by adding one of several auxiliaries. The fungicidal effect of a sulphur deposit depends upon the concentration at which the sulphur is applied, its particle size, the extent to which it is distributed on the plant, and its adhesiveness.

The committee on standards of the German Plant Protection Service has drawn up provisional standards for the approval of wettable sulphur preparations to avoid the necessity of having to carry out a biological test when new applications are made for preparations (Zeumer, 1949; Zeumer and Fischer, 1951). The firms manufacturing wettable sulphur have pointed out, in reply to this, that a final standardization of wettable sulphur preparations is not possible, and in fact not advisable, until it has been definitely established whether, and to what extent, the effect of wettable sulphur preparations is dependent upon those of its properties which can be determined quantitatively by physical methods. This means that it is the relations existing between the way in which the sulphur is prepared and its fungitoxic effectiveness which have to be examined in particular, whereby the initial effect and the persistence are factors of special importance to practical application.

Studies on these relations may furnish important information both for the planned standardization of the wettable sulphur preparations as well as for indicating the most suitable method of manufacturing the preparations. In addition such information would allow predictions to be made as to what effects the sulphur will have on the parasite and the host under different climatic conditions so that a better assessment can be made of the possibilities and limits of the application of wettable sulphur.

To investigate the questions in the laboratory, a satisfactory testing method is needed. Therefore, in Sections C and D the technical and biological prerequisites for the spore-germination test on slides will be discussed first. Section E is devoted to a description of the studies of the fungitoxic properties of wettable sulphur whereby fungitoxicity is understood to be the sum of the fungistatic and fungicidal properties.

B. Statistical methods of evaluating test results

The results were evaluated statistically according to the methods of Snedecor (1946), Weber (1948), Linder (1951) and Finney (1952). The probability P was computed by the analysis of variance procedure and in the t -test as a special case of the analysis of variance:

$P > 5\%$ = 0 = two means are variable only at random,

$P < 5\%$ = $*$ = difference is weakly significant,

$P < 1\%$ = $**$ = difference is significant,

$P < 0.1\%$ = $***$ = difference is highly significant.

Key to the signs used in the statistical data:

$\bar{x}$ = arithmetic mean,

$\bar{x}$ (ng %) = arithmetic mean of ng % values,

$\bar{x}$ (tr.) = arithmetic mean of arc-sin-ng % values according to Bliss,

$s_{\bar{x}}$ = standard deviation of mean,

N = no. of individual values (in germination tests, 50 spores are counted for each individual value).

n = no. of degrees of freedom,

SQ = sum of squares,

s^2 = mean square,

t and F = factors for determining the probability (cf. Linder, 1951, P. 225—228),

t' = factor for determining P according to the approximate method of Cochran and Cox,

LSD = least significant difference for $P = 5\%$, $P = 1\%$ and $P = 0.1\%$ (LSD_5 , LSD_1 , $LSD_{0.1}$).

Analyses of variance and t -tests may only be applied when the mean squares (s^2) of the spot tests are homogeneous. The homogeneity of variances was checked according to the χ^2 method (Snedecor, 1946, P. 188 ff.) and the F test (Linder, 1951, P. 96 ff.). A detailed comparison of both methods is being prepared by Kosswig. Wherever the variations of the spot tests were

heterogeneous, the approximate method of Cochran and Cox (Snedecor, 1946, P. 83) was applied.

In cases of correlations between the mean and the variance, which preclude an analysis of variance, they were cancelled by converting the ng % values (percentage of non-germinated spores) into the corresponding arc-sin-ng % values according to Bliss (Snedecor, 1946, P. 447 ff.). As absolute ng % values apply only to the test conditions described in this paper and to the biological material used in the tests, a separate calculation of $\bar{x}$ from the germination actually observed was not made in most cases.

The analysis of variance was carried out with multiple classification. The calculation of the interactions was primarily used for determining the discrepancy. They are discussed in the individual tables only insofar as it was deemed necessary for formulating the question in the test concerned.

The dosage response curves were plotted according to the probit analysis of Finney (1952).

C. Studies on the technique of testing fungicides in the laboratory

Numerous methods have been published for testing the fungitoxic properties of chemical substances in the laboratory. In these methods, every effort is made in some form or other to reproduce the natural conditions prevailing in the practical application of fungicides. Either the fungus is offered favourable feeding conditions through cultivation on a nutrient medium to which the test substance is added before or after the inoculation — such methods are described by Manten, Klöpping and van der Kerk (1950), Thornberry (1950) and Miller (1952) — or special consideration is given to a reproduction of the conditions on the leaf such as they are when a prophylactic spray is applied against spores which settle on a coating of fungicide in a droplet of rain. This spore-germination test first referred to by Reddik and Wallace (1910) is mostly conducted on slides. Experience gained in the intervening period has led to the development of a standardized method, the slide-germination test (Peterson, 1941; Amer. Phyt. Soc., 1943). This test produces results in a very short time and enables the relation of parasite to chemical to be analyzed without making any allowance for the natural host. It is, therefore, used for testing the fungitoxic properties of wettable sulphur. Its results are influenced by the following factors:

1. The specific behaviour of the test organism towards the chemical substance.
2. The environmental conditions prevailing during the period of the test.
3. The method by which the chemical substance is applied to the slides.

The purpose of the spore-germination test is to establish the reaction of the test organism. The outside conditions, namely temperature, relative humidity, ventilation and the preparation of the slides for the application of the spray coating, can be standardized considerably. The best method for applying the test substance to the slides has been made the subject of the author's own investigations.

I. Known methods for the quantitative application of test substances on slides

There are different ways of combining the test substance and the spores.

In the drop test, a suspension or solution of the substance to be tested is mixed with the spore suspension. Drops of definite sizes are taken from this mixture and applied to slides, or the mixture is stored in micro beakers (Shafer, 1952). This method has several disadvantages. The dosage is inaccurate, this being unavoidable when a pipette is used. No allowance is made for the solubility of the substance from the spray coating which is exposed to natural conditions. Dependent upon the percentage of those particles which are smaller than 1μ and suspended in water, a varying quantity of test substance will come into contact with the settling spores.

In the immersion method, slides are dipped in test substance solutions or suspensions of different concentrations. Drops of a spore content are applied to equal surfaces of the dried-on coating. According to Horsfall (1945, P. 20), this method is not suitable because the quantity of substance adhering to the slide varies with the surface tension of the solution.

The application of a fungicide suspension or solution by means of a pipette on to a defined surface on the slide has been described, inter alia, by Montgomery and Moore (1938), and Kirby and Frick (1953). In this method, inaccuracies may result due to fast sinking of the coarse particles during the pipetting process, and due to decomposition or losses of the substance when the coating dries, particularly with volatile substances (Doran, 1922).

Applications of fungicide to the slide, which quantitatively are most exact, are rendered possible by making use of the many spraying methods which are based on the field application of the fungicides. In the field the wash must be sprayed and distributed over a distance of several metres, this being done by using a special pressure nozzle with water as the vehicle. In order to change a spray jet into a fine spray mist by means of a nozzle, a pressure is required which for laboratory purposes is too high. In addition, the force of the impact caused when spray wash hits the slide would also be of disadvantage. If, on the other hand, air were to be used as the vehicle of the spray wash, a finer spray mist could be produced involving a lower pressure and smaller quantities of spray wash. The mist would then settle on the slide. It is essential that the quantity settling on the unit of surface is equal in each test, when the same substance is used at the same concentration.

When hand atomizers are used, the spray wash is not distributed evenly in most cases due to the unavoidable subjective influence caused by the movement of the atomizer and by the production of pressure with the rubber ball. Moreover, the quantity of wash applied per unit of surface varies, too.

The exposure factor varies among the many sprayer types fitted with stationary nozzles. McCallan and Wilcoxon (1940a) developed the "Settling Tower" in which the slide is exposed to a sinking spray mist for a definite period. The variations in the quantities of substance are quoted by these authors at 4.5 % for sprays carried out at the same time, and at 12.8 % for sprays applied at different times (McCallan and Wilcoxon, 1940b). With the "Horizontal Sprayer" described by Horsfall et al. (1940), a spray jet is directed horizontally on to a slide. The disadvantages of the "Settling Tower" and the "Horizontal Sprayer" are similar to those found in the apparatus recently constructed in Switzerland by Cifferri and Zobrist (Blumer and Kundert, 1950), who combined the principles of the "Settling Tower" and the "Horizontal Sprayer". By means of a horizontally set nozzle

with an adjustable angle, a fine spray cone is produced which is directed on to a sloping glass disk. The rough particles which are mostly carried farther by their weight, strike the disk and trickle down it. The fine droplets of the spray must, however, describe a short downward curve. They do not reach the disk and fall to the bottom of the spray chamber through an opening which can be closed by means of a cover. Below this opening, the slides are fitted on a rotating disk so that they are constantly moving past and the fine spray mist can settle on them. The exposure time for the slides can be varied by means of the hinged cover.

It was my intention to use this apparatus for my tests with wettable sulphur but several disadvantages came to light which would have greatly affected the results. To increase the evenness of the spray coating and to reduce the variations in the quantities of wash reaching the slides, the rough particles are filtered out of the spray cone. In actual fact, however, the spray cone underwent constant variations during spraying even with a permanent nozzle setting and equal air pressure. There was a poor supply of wash from the

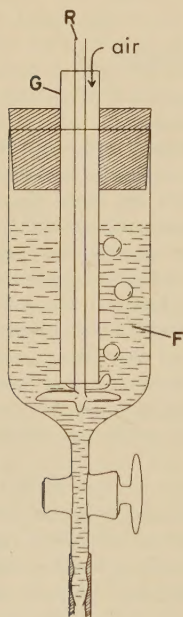


Fig. 1: Device for keeping constant the column of water above the spray nozzle.

F = Suspension or solution
of fungicide
G = Glass tube
R = Stirrer

container located above the nozzle because air frequently entered the feed pipe. This air was difficult to remove and consequently impaired the flow of wash. The level of the column of water above the nozzle caused a variation in the quantity of wash discharged by the nozzle. To avoid the necessity of having to continuously top up the wash, I closed the container with a stopper (Fig. 1) through which a glass tube was inserted running to the bottom of the container just above the outlet. Whenever wash now flowed out of the container a reduced pressure developed in the space between the rubber stopper and the level of the liquid. This reduced pressure was balanced by the inserted glass tube so that the column of water above the outlet never dropped below the bottom edge of the glass tube while the wash was flowing out. This device, however, was always ineffective when air was enclosed in the feed pipe leading to the nozzle because as a result the column of water was suspended. These disadvantages are accompanied by two others which restrict the use of the above-mentioned apparatuses in laboratories where many new substances have to be tested daily:

1. Very often only small quantities of new substances can be sent in at first for the biological test. Due to the large consumption of wash in the test, however, the quantities of substance required are too large.
2. Mostly several preparations have to be tested at different concentrations and in two or three replications. Changes of spray wash

and cleaning of the apparatus before a new preparation is tested should, therefore, involve only a slight loss of time but this is seldom the case.

Therefore, an attempt had to be made to overcome these disadvantages by developing a new apparatus to meet the following requirements:

1. Simple construction combined with easy and safe handling of the apparatus.
2. Constancy of the quantity of spray wash reaching the slide.
3. Production of an even spray coating.
4. Satisfactory stirring action and low consumption of wash.
5. Time taken for changing the spray wash and cleaning the apparatus to be reduced to a minimum.

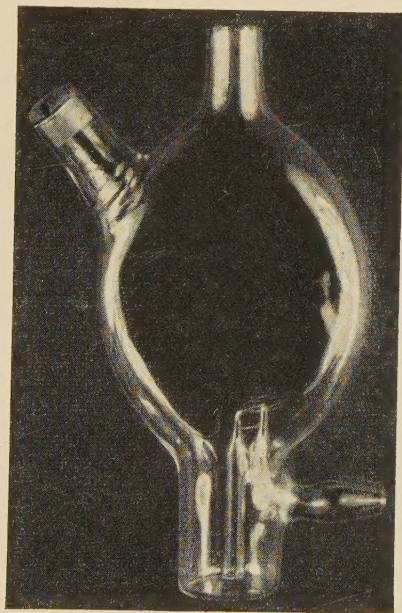


Fig. 2: Spray container for the pendulum sprayer.

II. Author's own studies on the quantitative application of fungicides on slides with the aid of a newly developed sprayer

1. Construction and handling of the apparatus

Several nozzles were tested for their efficiency to produce an even spray mist. Nozzles which operate on the principle of the air-blast machine, as used for example by Cifferri and Zobrist, have two disadvantages:

1. The spray cone is irregular and mostly it is compressed somewhat in the centre so that when the spray jet is directed on to the slide, spray paths or spray spots of unequal density may be produced.

2. The consumption of wash is mostly very high because only a very small portion of the ejected spray wash actually reaches the slide.

Nozzles working on the principle of fixative sprays proved very efficient (cf. Evans and Martin, 1935) but the consumption of wash is very high even with these types.

A glass container with a built-in nozzle working on the latter principle, which was made to produce inhalation mists, promised good results. In this apparatus, the spray wash only comes into contact with glass but not with rubber or any other feed pipe material. By directing the spray jet inside the inhalator on to a point next to the opening, most of the liquid is made to remain in the container. Only a small part of the wash is able to escape upwards through the opening in the form of a very fine spray mist (Fig. 2).

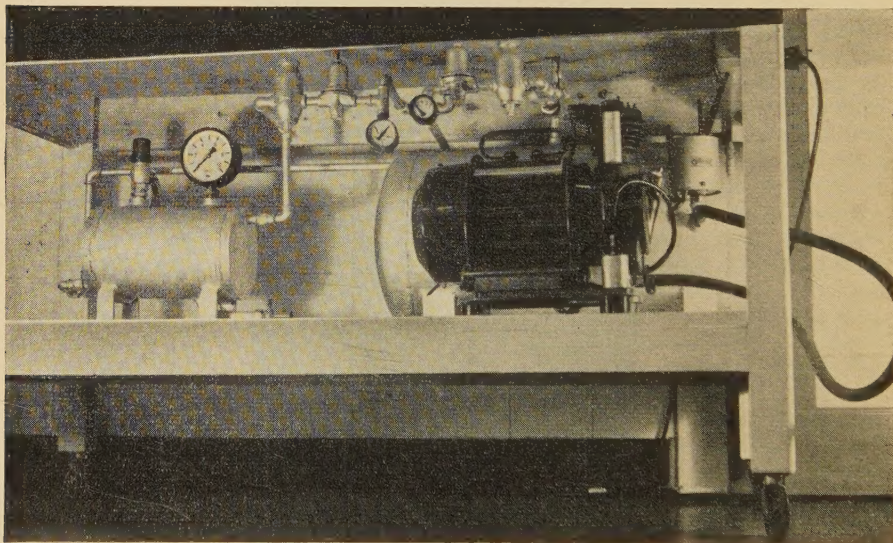


Fig. 3: Plant for producing and regulating the air pressure.

The fineness of the mist depends upon the pressure at which the spray wash is atomized. It must, therefore, be adjusted exactly, this being done as follows (Fig. 3):

Air is compressed in a tank to a pressure of 3 atmospheres gauge. The pressure fluctuation in this tank amounts to two atmospheres because once the pressure has dropped to 1 atmosphere gauge, the compressor automatically comes on again until 3 atmospheres gauge are attained. These fluctuations are eliminated for the sprayer itself in that by reducing the pressure to 1 atmosphere gauge the air is led from the main tank into a second pressure vessel. The air leaving this second pressure vessel at a uniform pressure of 1 atmosphere gauge is reduced to 0.5 atmosphere gauge by a second pressure-reducing valve, and fed via a needle valve. Consequently, the pressure needed for the sprayer can

be kept constant, and can also be adjusted via a mercury gauge without being influenced by the fluctuations in the first tank. The best pressure for carrying out spraying tests with the above-mentioned inhalator has been found to be 70 ± 0.5 mm Hg.

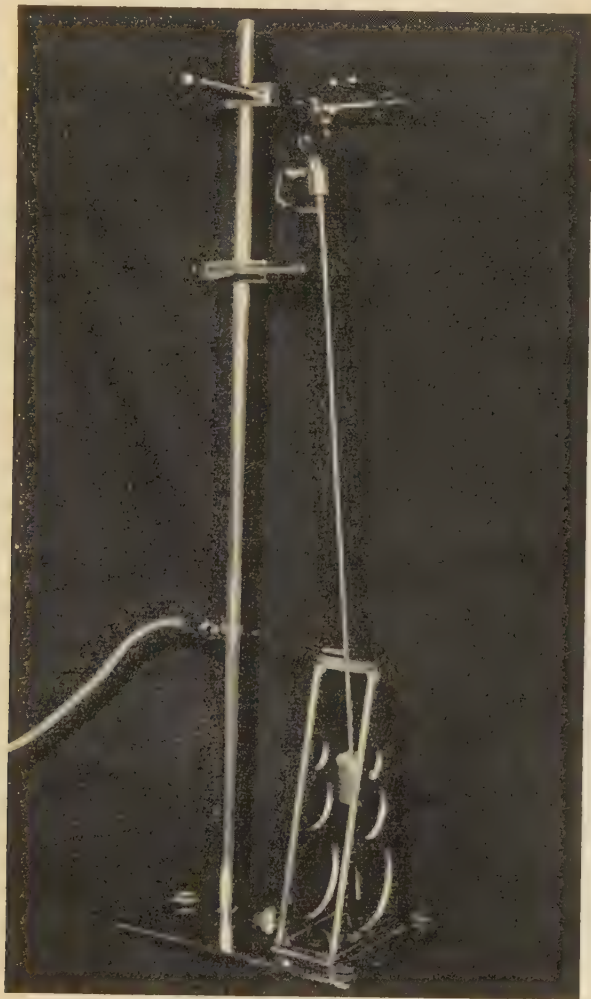


Fig. 4: Pendulum sprayer.

The quantity of spray wash required, the fineness of the spray mist and the coverage of the mist are factors all determined by the pressure. The diameter of the spray cone is limited by the width of the inhalator opening, amounting to approximately 9 mm at a distance of 30 mm between the edge

of the opening and the slide. Consequently there are two requirements to be fulfilled:

In order to cover the entire width of the slide, two paths must be sprayed on the slide with a definite spacing between them at the given diameter of the spray cone. The sprayer must, therefore, be moved along underneath the slide without causing any lateral deviations.

The distance from the edge of the inhalator opening to the slide must be equal over the entire length of the slide to ensure that the width of the impinging spray jet and the quantity of spray wash reaching the slide remain constant.

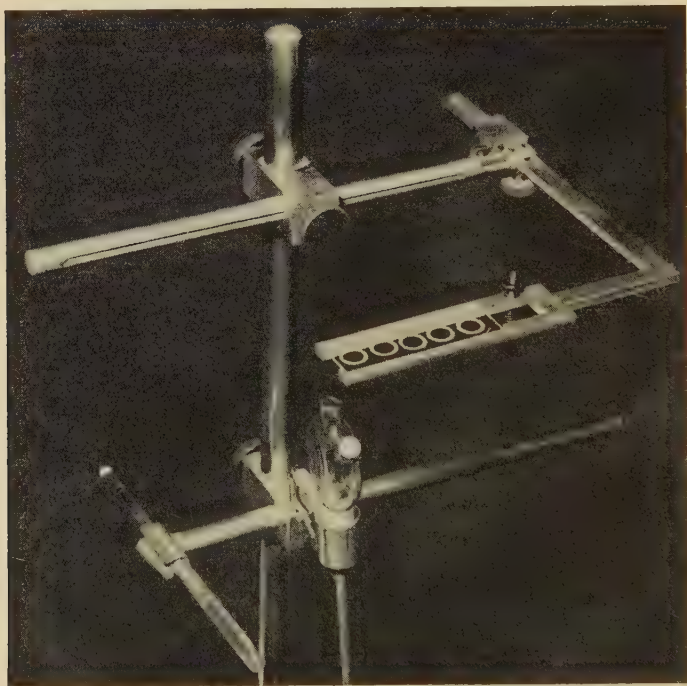


Fig. 5: Partial view of the pendulum sprayer.

This can be done by attaching the spray container to the upper end of a long pendulum (see Fig. 4).

As the pressure, the quantity of wash in the glass container and the exposure of the slide to the path of the vibrating spray container can all be kept constant, it is merely the number of vibrations and the speed of the pendulum that determine the quantity of spray wash on the slide. A glass rod, guided in a glass tube, is used to arrest the movement of the pendulum. The glass rod can be moved in front of the pendulum so that the latter is always released from the same starting position.

The apparatus is easy and safe to handle. The glass container is located on the top of the pendulum with the base inserted in a cup and connected to the pressure line. The slide is located on a bracket fitted above the pendulum (Fig. 5).

2 ml of spray wash are pipetted through the top opening into the container. When filling, care must be taken that the nozzle is not wetted before spraying and that no drops are left on the edge of the opening otherwise the slides sprayed first will receive more wash than the others. After the tap has been opened on the pressure line, the pressure is adjusted at the Hg-gauge. By drawing back the glass rod, the pendulum is released and after making four vibrations it is stopped again and locked in position. The slide is turned

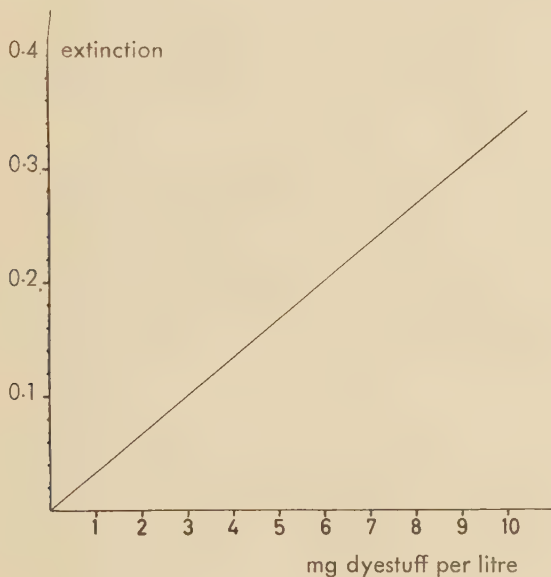


Fig. 6: Calibration curve for benzo fast scarlet 4 BAS.

horizontally through 180°, replaced and exposed again to the spray jet for four vibrations. As a result two spray paths are directed on to the slide. These paths intersect so that there is just as much coating of spray in the centre as at the edges. In this way, eight slides can be sprayed with one filling.

2. Constancy of the quantity of wash reaching the slide

In view of the very small quantity of substance required per slide, it appeared impossible to determine for sulphur the constancy of the quantity of wash reaching the slide by means of a volumetric analysis, such as recommended, for example, by Ehlers (1953a) in respect of somewhat larger surfaces. On the other hand, there appeared to be every prospect of obtaining good results by using a dyestuff which, photometrically, permitted the determination of the quantity of substance and liquid actually

reaching the slide. 0.001 % to 0.0001 % solutions were prepared with the water-soluble dyestuff "Benzoechtscharlach 4 BAS" (benzo fast scarlet 4 BAS). The extinction values for these solutions were measured by using the Hg 546 filter in the Eppendorf photometer of Messrs. Medeor, Hamburg. These values gave a straight-line curve (Fig. 6).

The dyestuff was then sprayed on to the slide in a solution of 1 %. The dried-on coating was washed off quantitatively with 10 ml of water, and the extinction value (E) was determined for this solution.

The quantity of wash reaching the slide can be calculated from the initial concentration of the sprayed dyestuff (1 %) and the quantity of dyestuff found according to the E-values. The average of 25 measurements amounted to 9.9 mm^3 ($\bar{x}$ [E] = 0.333 ± 0.0016 or 99 gamma dyestuff). This value corresponds to a quantity of 0.087 gamma dyestuff or 0.0087 mm^3 spray liquid per mm^2 for a sprayed surface of $76 \times 15 \text{ mm} = 1140 \text{ mm}^2$. By inserting the initial concentration e.g. of a sulphur preparation in an equation, the quantity of sulphur sprayed per unit of surface (mm^2) can be calculated from the given quantity of spray liquid per mm^2 .

The spray coating on slides which, in addition to being cleaned as usual in chromosulphuric acid, water and alcohol and subsequently dried at 150°C , were then wiped with a leather cloth, had a different appearance from a coating on slides which had not been wiped. The droplets on the wiped slide were smaller because they ran together less, this presumably being due to the fact that by using the leather cloth, a small amount of fat was applied to the slide. The following extinction values, which were obtained after spraying 1 % solutions and washing off the slides with 10 ml of water, show that the wiping also influences initial adhesiveness (Magie and Horsfall, 1936):

Table 1: Influence of the wettability of the slide upon the quantity of spray coating (specification of the extinction value E).

	N	$\bar{x}$ (E)	$s_{\bar{x}}$	t' (14)
Slide wiped	15	0.321	0.0016	6.02 ***
Slide not wiped	15	0.346	0.0040	

As the deviation was less on wiped slides ($s_{\bar{x}} = 0.0016$), all the slides were wiped with a leather cloth washed out in acetone before they were used.

3. Uniformity of the spray coating

In order to check the uniformity of the spray coating on the whole of the sprayed surface, two series of tests were carried out:

The distribution of the spray wash was examined over the length of the slide by evaluating one third each of the sprayed surface separately after breaking up the slide which had previously been scored for this purpose.

Table 2: Distribution of the spray wash over the length of the slide (specification of the extinction value E).

Slide	Section	E	Length of glass expressed in mm	E per $25 \times 18 \text{ mm} = 450 \text{ mm}^2$
1	a	0.140	25.5	0.137
	b	0.136	25.0	0.136
	c	0.143	26.0	0.137
2	a	0.140	25.5	0.137
	b	0.128	24.3	0.132
	c	0.138	25.5	0.135
3	a	0.135	25.0	0.135
	b	0.135	25.0	0.135
	c	0.140	26.0	0.135
4	a	0.138	25.5	0.135
	b	0.133	25.0	0.133
	c	0.136	25.0	0.136

The maximum deviation, $E = 0.132$ to $E = 0.137$, amounted to 3 gamma dyestuff per 450 mm^2 .

The distribution of the spray wash over the breadth of the slide was determined as follows:

Strips of adhesive tape were stuck on the slide so that a path 5 mm wide was left free either in the middle or towards the edge. After spraying, the strips were peeled off, and the dyestuff was washed off from the exposed parts of the slide with water. As it was difficult to mark off the surface exactly by sticking on the strips, there may be variations in the quantity of wash.

Table 3: Distribution of the spray wash over the breadth of the slide (specification of the extinction value E).

	N	$\bar{x}$ (E)	s^2_x	
Centre strips	11	0.198	0.0031	$\text{LSD}_5 = 0.007$
Periphery strips	11	0.190	0.0017	$\text{LSD}_1 = 0.010$

The difference is just significant at $P < 5 \%$. As the drops of the spore suspension were always placed exactly in the centre — the prerequisites, therefore, being the same in every test with respect to this small overdosage in the centre — the degree of exactitude sufficed for my investigations. The differences could have been eliminated by even more exact adjustments.

4. Effect of stirring and consumption of wash

The tests described so far were carried out with solutions. It was my intention, however, to spray suspensions of sulphur of different particle sizes in my tests. Therefore, a check had to be made on the effect of stirring. As the wash in the vessel is sucked in from the bottom, although a large part flows back from the top, it was to be assumed that a special stirring process would not be necessary. The following test was carried out to check the correctness of this assumption:

A small quantity of the dyestuff Ceresrot 3 R, which is insoluble in water, was dissolved in toluene adding 0.5 ml of an oil-soluble emulsifier. This solution was stirred and emulsified in water. After a long period of stirring, the droplets of dyestuff + toluene + emulsifier emulsified in water were smaller than 20 μ and 6 μ respectively. The following E-values were obtained with these emulsions upon spraying:

Droplets smaller than 20 μ E	Droplets smaller than 6 μ E
0.198	0.170
0.192	0.176
0.194	0.177

According to these values and the results obtained with sulphur fractions which have yet to be discussed (Section E II), the stirring effect may be regarded as satisfactory.

As it is always only a small part of the wash boosted by the nozzle that actually leaves the opening, which means that the greater part flows back, it may be concluded that the consumption of wash is very slight.

5. Time involved

Whereas it takes approximately one hour to clean the sprayer of Cifferri and Zobrist, only a fraction of this time has to be spent on cleaning the apparatus described above.

III. Method employed in carrying out the spore-germination test on slides

I selected the following method for the investigations with the newly developed pendulum sprayer:

5 paraffin rings, 9.5 mm in diameter equal to 354 mm² total area, were placed on each slide after they had been carefully cleaned. The slides were then sprayed with the test substance. Mostly two slides were sprayed with each concentration. Drops of spore suspension with a content of 0.05 ml were placed on the dry coating of fungicide (cf. Montgomery and Moore, 1938). The spore density in these drops was adjusted so that approximately 50 spores were counted on a marked out surface of 650 $\times$ 650 μ at 120 $\times$ magnification. This corresponds to a content of 150,000 spores per ml according to measurements taken in a hemocytometer (blood-counter chamber) designed by Thoma. The

uniformity of the drops was attained by means of a specially constructed apparatus (Fig. 7).

The spore suspension trickles out of an opening in a vessel. Washed air flowing through at a slow rate ensures the uniform distribution of the spores. The suspension collects beneath the opening in a staple, and breaks up into drops of equal sizes. To avoid water collecting on the glass and thus causing the drops to increase in volume, the glass surface around the staple is rendered water-repellent with dimethyl dichlorosilane vapours.

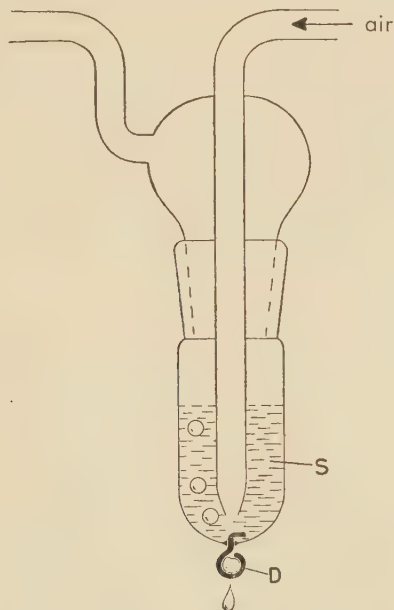


Fig. 7: Apparatus for applying spore suspension drops of equal sizes to slides.
D = Nichrome wire; S = Spore suspension

0.05 ml of spore suspension reached a sprayed surface of 70.8 mm². The spore concentration of the suspension amounted to approximately 150,000 spores per ml in all my tests. For example, when 0.1 % wettable sulphur was sprayed, approximately 7,500 spores came into contact with about 0.6 gamma wettable sulphur in one drop. This quantity of wettable sulphur is calculated as follows according to the determinations of the wash consumption: 0.0087 mm³ of wash covers 1 mm² of surface. At an initial concentration of 0.1 %, this corresponds to 0.0087 gamma wettable sulphur per mm² surface. Consequently $70.8 \times 0.0087 = 0.61596$, i.e. approximately 0.6 gamma wettable sulphur covers a surface of 70.8 mm². In this way, the quantities of fungicide applied per unit of surface in the test can be calculated quite easily from the concentrations expressed in % which are given later on.

D. Studies on the biological prerequisites for the spore-germination test with conidia of *Venturia inaequalis*

I chose *Venturia inaequalis* (Cke.) Wint. for carrying out the biological test of the fungitoxic properties of sulphur, firstly because wettable sulphur has some practical significance for the control of this parasite, and secondly because it can be bred on artificial nutrient media all the year round. Moreover, it is sufficiently sensitive to sulphur also for slight differences in effectiveness to be recorded.

Keitt and Jones (1926), K ü t h e (1935), J a h n (1943a) and others have published comprehensive data on the culture of *Venturia inaequalis*. As great difficulties are encountered in obtaining a uniform ascospore material during the whole year (Keitt and Langford, 1941; J a h n, 1943b), I made exclusive use of conidia. To carry out the spore-germination test, it is essential that spores are available in large quantities, that a high percentage of them germinate, and that their germination remains uniform in consecutive tests. A check had to be made, therefore, to establish whether and to what extent the test object would fulfil these requirements.

I. Data extracted from pertinent literature

Venturia inaequalis can be cultivated on several kinds of solid and liquid nutrient media. Rudloff (1935a and b) selected from a large number of nutrient media what he considered to be the most suitable. He found that the sporulation and the germinating capacity of conidia are influenced by the nutrient media. But the mycelium growth and sporulation need not always proceed parallel to each other. For example, in Pulst's nutrient solution the growth of mycelium was very good whereas the production of conidia was unsatisfactory. Peptone used as the N-source delayed the production of conidia. Raw sugar as the C-source gave a high production of conidia at concentrations of up to 1 %, but inhibited the production at higher concentrations.

J a h n (1943a) established that the spore yield is dependent upon the concentration of the nutrient medium. Due to less germination, there were more conidia on 80 % beer wort agar than on 2 % malt agar.

The influence of the pH value on the growth of the fungus *in vitro* was examined by Wiesmann (1931), K ü t h e (1935), J a h n (1943a) etc. According to these authors, most single-spore strains thrive best at pH = 7.

With certain fungi it was found that the age of the culture influences the conidial yield and the spore germination (McCallan, 1930). Miller (1949) studied this question closely with *Venturia inaequalis*. He found that the development of conidia on 5 % malt agar at room temperature (approx. 21 °C) increased greatly from the fourth day onwards (cf. Aderhold, 1894), then reached its maximum after seven days, only to decrease again later. The germinating capacity of conidia is also influenced by the age of the culture (McCallan, 1930; McCallan and Wilcoxon, 1941). In replicated tests,

Miller (1949) showed that the germinating capacity decreases considerably as the age of the culture increases, an observation which was confirmed by Hartsuiker (1940).

Extensive investigation results are on record with respect to the influence the temperature exerts on the fungus. Aderhold (1900), Keitt (1926), Keitt and Jones (1926), K ü t h e (1935), Winkelmann and Holz (1936), Hartsuiker (1940) and J a h n (1943a) all established that 18 to 20 °C is the optimum temperature for the mycelium growth. At temperatures of over 24 °C, the growth is strongly inhibited, and at 30 °C the fungus is killed (J a h n, 1943a). Rudloff (1935a) found that the mycelium stopped growing at 26 °C. On the other hand, the mycelium growth did not stop completely even at temperatures as low as 0 °C (J a h n, 1943a).

McCallan (1930) and McCallan and Wilcoxon (1941) showed that the temperature influences the germination both during the formation of conidia as well as during the spore-germination test. According to McCallan (1930), the optimum temperature for conidial formation differs from that for germination. The conidial formation, according to Miller (1949), reaches an optimum in seven day old cultures at 21 °C, and in 25 day old cultures at 9 °C. He also observed that the influence the temperature exerts upon the germinating capacity of the conidia during the breeding of the culture is dependent upon the age of the culture. Cultures of conidia kept 7 to 14 days at 18 °C, 11 to 14 days at 15 °C and 11 to 18 days at 12 °C all attained 90 % germination. J a h n (1943a) found that maximum conidial germination took place at 20 to 22 °C, whereas it was weak at 27 °C, strongly inhibited at 30 °C, and at 35 °C it was very seldom that a few conidia germinated with a short germ tube. At temperatures as low as 2 °C, it was not the percentage of germination but only length of the germ tube which was reduced (cf. Aderhold, 1900).

The influence of humidity upon the germination of *Venturia inaequalis* conidia was studied by Clayton (1942). He obtained maximum germination only at 100 % relative humidity. At 99 % relative humidity, germination dropped to approximately 50 %, and at 98 % relative humidity there was no germination at all. Observations made in the field (Mills and Laplante, 1951) also support this, in that they showed leaves must be wet for at least seven hours at optimum temperatures (20 to 22 °C) for the conidia to germinate strongly enough. As the spores suspended on slides in water are kept in Petri dishes in the spore-germination test, this factor can be neglected.

Investigations carried out by D o r a n (1922), McCallan (1930) etc. showed that the conidia of *Venturia inaequalis* are not dependent upon light for their germination.

II. Author's own investigations with conidia of *Venturia inaequalis*

1. Studies on conidia from mass-spore cultures

In the Spring of 1951, eight ascospores of one tube were obtained from a perithecium of a leaf of the James Grieve apple variety. These were germinated on 10 % beer wort agar and then further bred for a time on the same nutrient medium. Experience has shown that during the course of time, under

certain conditions after only a few months, cultures of *Venturia inaequalis* may lose their power of being able to produce conidia (Hartsuijker, 1940). This observation was also made on the above-mentioned population. While being bred on beet wort agar, the sporulation decreased so greatly that in the end sufficient spores could no longer be obtained. By changing over to another nutrient medium — Pulst's nutrient solution (1902) varied with 40 g of raw sugar + 0.04 g of aminoacetic acid + 1 g of potassium dihydrogen phosphate + 0.15 g of potassium nitrate + 0.15 g of magnesium sulphate per litre of water — the sporulation was stimulated again. This led to the fungus being bred alternately on 10 % beer wort agar and in Pulst's nutrient solution.

In order to establish what influence the change from solid to liquid nutrient medium has on the sporulation and percentage germination of conidia, I inoculated daily six flasks of Pulst's solution and six agar slant cultures. After seven days of growth, spores were transferred to fresh nutrient medium in the manner explained in Table 4. In 13 tests, 500 spores were taken for each spore-germination test from 6, 7 and 8 day old cultures in the following combinations:

1. From Pulst's solution inoculated with spores of agar.
2. From Pulst's solution inoculated with spores of Pulst's solution.
3. From agar inoculated with spores of Pulst's solution.
4. From agar inoculated with spores of agar.

Table 4: Influence change of nutrient medium has on conidial germination.
N = 13.

Culture was inoculated from	Spores of culture	Age of culture (days)	$\bar{x}$ (ng%)
1. Agar	in Pulst's solution A ➡ ➡ P	8	7.4
		7	6.1
		6	6.9
2. Pulst's solution	in Pulst's solution P ➡ ➡ P	8	14.2
		7	11.0
		6	13.1
3. Pulst's solution	on agar P ➡ ➡ A	8	12.8
		7	12.7
		6	13.1
4. Agar	on agar A ➡ ➡ A	8	22.4
		7	16.3
		6	23.1

In the statistical evaluation of the results, the arc-sin-ng % values according to Bliss (Snedecor, 1946) were inserted for the individual values (ng %). The three x-values of one culture type were combined to give one arithmetic mean.

Table 4a: Influence change of nutrient medium has on conidial germination.

Type of culture	N	$\bar{x}$	$s_{\bar{x}}$	
1. (A $\rightarrow$ P)	13	14.75	0.621	
2. (P $\rightarrow$ P)	13	20.73	0.986	LSD ₅ = 2.72
3. (P $\rightarrow$ A)	13	20.72	1.098	LSD _{0.1} = 4.74
4. (A $\rightarrow$ A)	13	26.86	1.042	

Culture types 2 and 3 do not differ. All other differences are significant at $P < 0.1$ %.

According to the results, a constant change of nutrient medium, and the removal of the conidia from the liquid nutrient medium, creates the best conditions for the germination. The fungus was, therefore, cultivated on 2 % malt agar in slant culture and in Pulst's nutrient solution in Erlenmeyer flasks. The nutrient medium was changed every seven days by transferring small pieces of agar with abundant spore-bearing mycelium into the nutrient-containing liquid and then shaking well or, alternatively, by shaking the nutrient solution thoroughly and then pouring small quantities of the culture over agar slant cultures.

As there were sometimes large variations in germination during the test, I made a check to determine whether and to what extent reproducible results can be obtained with conidia from mass-spore cultures. Three flasks of Pulst's solution were inoculated in one day from an agar slant culture of the above-mentioned population. After seven days, conidia were taken from these Pulst's cultures, and germinated in distilled water and on spray coatings of a commercial colloidal sulphur. The method selected for doing this is described in Section C, III.

Table 5: Germination of *Venturia inaequalis* conidia from three cultures of a population in Pulst's nutrient solution, when treated with a commercial colloidal sulphur. Spores from 7 day old cultures. N = 5.

	Culture 1		Culture 2		Culture 3	
	$\bar{x}$ (ng ⁰ /o)	$s_{\bar{x}}$	$\bar{x}$ (ng ⁰ /o)	$s_{\bar{x}}$	$\bar{x}$ (ng ⁰ /o)	$s_{\bar{x}}$
Check	11.4	1.15	11.8	1.14	9.2	1.08
0.12 % sulphur	96.8	0.65	94.8	1.64	64.0	1.54
0.24 % sulphur	98.6	0.67	96.6	1.72	89.0	1.70
0.48 % sulphur	99.6	0.45	94.6	0.76	92.0	1.06

LSD₅ = 3.1

LSD₁ = 4.1

LSD_{0.1} = 5.4

Table 5a: Analysis of variance to Table 5.

	n	SQ	s ²	F
Total	59	78,833		
Culture (C.)	2	1,958	979	168.4 ***
Treatment (Trea.)	3	74,748	24,916	4,286.6 ***
C. × Trea.	6	1,848	308	53.0 ***
Remainder	48	279	5.81	

The test shows that spores from a mass-spore culture, which do not differ in their germinating capacity, may react differently on sulphur even when the flasks of nutrient solution are inoculated on the same day from the same culture, and are cultivated under equal conditions. This becomes most evident on comparing cultures 1 and 2 with culture 3. The only explanation that could be given for this finding was that the percentages of sulphur-shy and sulphur-resistant strains in one population may vary from culture to culture. I carried out tests with single-spore cultures to confirm this assumption.

2. Studies on conidia from single-spore cultures

In the culture of single-spore strains, Palmiter (1934), Rudloff (1935b) etc. found that several scab strains may differ greatly with respect to the production and germinating capacity of conidia. Keitt and Palmiter (1938) established that out of the cultures bred from eight ascospores of one tube, each four behaved similarly with respect to their sexual compatibility and pathogenicity (cf. Keitt and Langford, 1941; Langford and Keitt, 1942; Keitt, Langford and Shay, 1943). The heterothallism expressed therein, which was already presumed by Killian in 1917, may of course also appear in variable growth on artificial media and in morphological characteristics. A variation in the resistance of different scab strains to chemicals was established for the first time by Palmiter (1934) in tests with copper sulphate and Paris green. In certain cases he encountered considerable differences, although he only observed the mycelium growth of the fungus on malt agar to which the toxic substances had been added in given concentrations. McCallan and Wilcoxon (1939) examined the latter problem in an analysis of factors causing variation in spore-germination tests on slides. Their investigations were carried out with copper sulphate on spores of *Sclerotinia fructicola* (Wint.), and did not reveal any reaction of single-spore strains to chemicals.

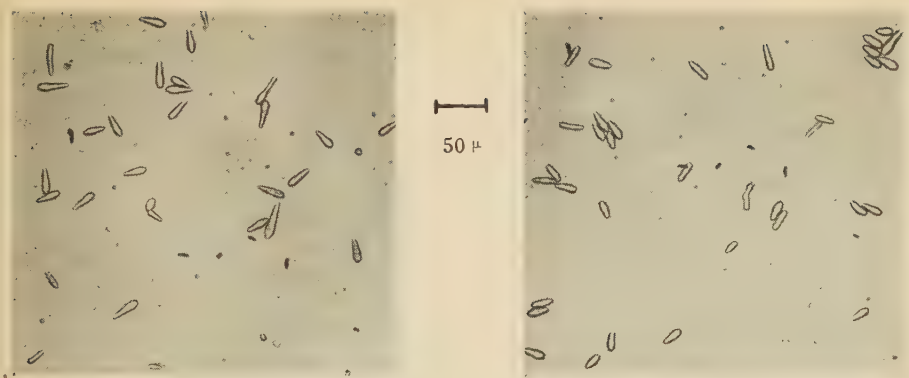


Fig. 8: Conidia of the single-spore strains EK 4/53 (Type A) and EK 7/53 (Type B).

a) Morphological and physiological differences among self-bred single spore strains

In 1953, eight single conidial cultures (EK 1—8/53) were prepared from the population obtained in 1951, and a check was kept on their development. It was found that the eight strains could be classified in two clearly distinguishable types, according to their different features.

The form of the conidia permitted a distinction to be made between the strains. The conidia of EK 7/53 (Type B) appeared somewhat more compact than those of EK 4/53 (Type A), as may be seen from Fig. 8.

Germination tests carried out in distilled water with conidia from seven day old cultures showed that conidia of Type A germinated at a higher percentage than those of Type B.

Table 6: Germination of conidia of different single-spore strains, expressed in %. 24 hours' germination in distilled water at $\sim 20^{\circ}\text{C}$.

	N	$\bar{x}$	$s_{\bar{x}}$	$t' (35)$
Type A (EK 2, 3, 4, 6/53)	36	96.4	0.17	19.5***
Type B (EK 1, 5, 7, 8/53)	36	88.6	0.48	

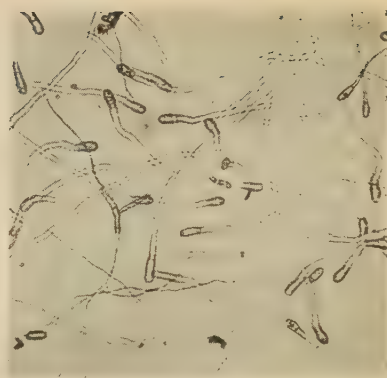
The difference in germination is significant at $P < 0.1\%$.

The conidia of Type A formed much shorter germ tubes than those of Type B. The former were partially branched off and developed adhesive organs directly on the spores (EK 4/53 = Type A), while the latter did not branch off and formed no adhesive organs (EK 7/53 = Type B), as shown in Fig. 9.



EK 4/53

50 μ

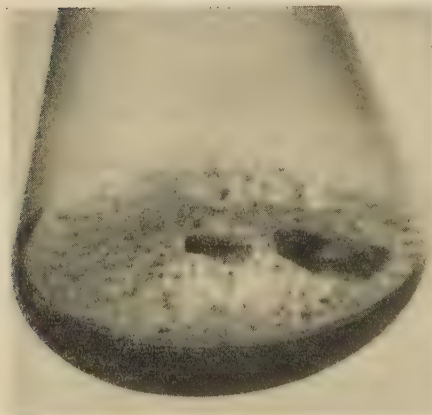


EK 7/53

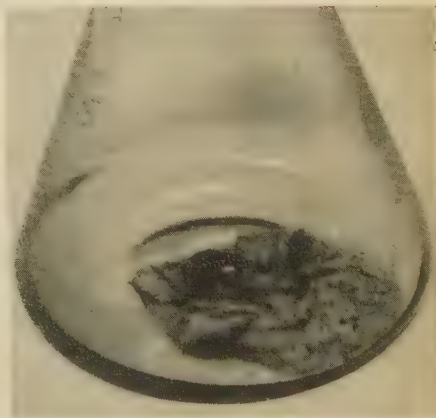
Fig. 9: Conidia of the single-spore strains EK 4/53 and EK 7/53 after 24 hours' germination in distilled water at $\sim 20^{\circ}\text{C}$.

Such variations in the development of the appressoria were already mentioned by Aderhold in 1896 and by Wiltshire in 1915. The formation of adhesive organs on only one of the two strains has the following effect. On cultures in Pulst's nutrient solution, EK 4/53 forms a finely flocculent mycelium covering which adheres to the bottom of the flask whereas EK 7/53 forms a well-branched mycelium covering which swims freely in the solution.

Germ tube measurements on conidia of EK 4/53 (Type A) and EK 7/53 (Type B) after 28 hours' germination in distilled water at $\sim 20^{\circ}\text{C}$ showed that



EK 4/53



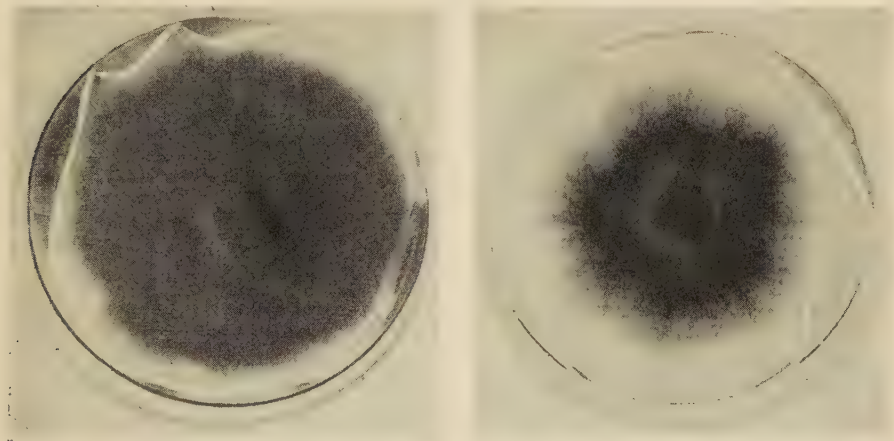
EK 7/53

Fig. 10: Cultures of the single-spore strains EK 4/53 and EK 7/53 in Pulst's nutrient solution after 14 days' growth at $\sim 20^{\circ}\text{C}$.

EK 4/53, determined as the average of each 100 counts, formed germ tubes $64.1 \pm 4.53 \mu$ long, while those of EK 7/53 were $299.4 \pm 9.07 \mu$ long ($t'_{(99)} = 23.217$; $P < 0.1 \%$).

Type A had a greyish-green mycelium covering on beer wort agar, while that of Type B was mouse-coloured. The colours were retained also in cultures of the strains on 2 % malt agar.

The rate of growth also varied greatly for both types. Cultures on beer wort agar in Petri dishes had a diameter of approx. 80 mm for Type A and approx. 50 mm for Type B (cf. Fig. 11) after six weeks' growth at a constant temperature of $20 \pm 0.2^\circ \text{C}$.



EK 4/53

EK 7/53

Fig. 11: Cultures of the single-spore strains EK 4/53 and EK 7/53 on beer wort agar after 6 weeks' growth at $\sim 20^\circ \text{C}$.

b) *The influence exerted by the age of the culture, temperature and light on sporulation and conidial germination of the strains EK 4/53 and EK 7/53*

EK 4/53 (Type A) and EK 7/53 (Type B) were used for investigating the variations in the physiological behaviour of different scab strains. To begin with, I checked the influence which the age of the culture has on the germinating capacity of the conidia of these two strains.

Conida from older cultures germinate worse. The age of the culture influences the germinating power of the conidia of both strains to a varying degree. As I found most of the conidia in the cultures in Pulst's solution after 7 days of breeding, I used conidia from 7 day old cultures in all other tests to eliminate the influence exerted by the age of the culture on the germinating capacity.

According to McCallan (1930), the temperature influences the germinating capacity of conidia while they are being formed. A check had to be made to establish whether the two strains differ in this respect and to what extent

Table 7: Conidial germination in distilled water in relation to the age of the culture for single-spore cultures.

24 hours' germination at $\sim 20^{\circ}\text{C}$. $N = 5$.

Age of culture expressed in days	EK 4/53 $\bar{x}$ (ng $^{\circ}/\text{o}$)	EK 7/53 $\bar{x}$ (ng $^{\circ}/\text{o}$)	Age of culture expressed in days	EK 4/53 $\bar{x}$ (ng $^{\circ}/\text{o}$)	EK 7/53 $\bar{x}$ (ng $^{\circ}/\text{o}$)
20	10.1	12.3	7	2.3	8.1
17	14.6	18.0	6	2.3	3.8
14	15.8	15.3	5	2.3	10.9
11	3.5	15.9	4	4.9	6.3

the yield of conidia is dependent upon the temperature. I cultivated the two strains EK 4/53 and EK 7/53 in Pulst's solution for seven days at 15°C , 20°C and 25°C , also at alternating temperatures by keeping them at 5°C and 10°C overnight (13 hours), and at 20°C during the day (11 hours).

The quantity of spores produced per culture was determined in the following manner. After centrifuging the contents of a flask and pouring off the excess nutrient solution, water was poured in until in one drop (0.5 ml) at $120\times$ magnification 50 spores were counted in the counting field of a crossline ocular ($650\times 650\text{ }\mu$). The absolute spore content per ml in a spore suspension thus adjusted amounts to approximately 150,000 spores. The figures listed in Table 8 in the column headed "No. of spores" when multiplied by 150,000 give the total spore content of a flask containing 15 ml of Pulst's nutrient solution.

Table 8: Sporulation and germinating capacity of the conidia of two strains when cultivated at different temperatures.

24 hours' germination in distilled water at $\sim 20^{\circ}\text{C}$. $N = 4$.

Temperature during cultivation		EK 4/53		EK 7/53	
		No. of spores (z) ¹⁾	$\bar{x}$ (ng $^{\circ}/\text{o}$)	No. of spores (z)	$\bar{x}$ (ng $^{\circ}/\text{o}$)
night	day				
5°C	20°C	5.5	6.0	6.3	2.2
10°C	20°C	3.9	6.6	8.7	2.1
	15°C	12.0	4.2	19.8	12.1
	20°C	21.5	4.6	20.7	11.4
	25°C	9.6	11.0	7.0	12.0

$\text{LSD}_5 = 2.7$

$\text{LSD}_1 = 3.6$

$\text{LSD}_{0.1} = 4.8$

¹⁾ No. of spores in 15 ml Pulst's nutrient solution = $z \times 150,000$.

It was found that the low night temperatures inhibited the sporulation of both strains. The maximum sporulation of EK 4/53 was at 20° C, and that of EK 7/53 at 15 to 20° C.

The ng % values have been determined from 40 individual values (4 tests with 10 counts each). In the analysis of variance of the results, the ten counts of one test were combined so that there were four individual values to each ng % value.

Table 8a: Germination of conidia exposed to different temperatures.

	n	SQ	s ²	F
Total	39	691.975		
Strains (St.)	1	24.025	24.025	6.948*
Cultivation conditions (Cult.)	4	302.850	75.713	21.895***
St. × Cult.	4	261.350	65.337	18.894***
Remainder	30	103.750	3.458	

The spores from cultures of EK 4/53 kept at alternating temperatures germinated just as well as those exposed to temperatures of 15° C and 20° C. In contrast, the cultures of EK 7/53 kept at low night temperatures contained relatively few spores with a very high germinating capacity. Therefore, both strains reacted to the temperatures by showing variations in their production of conidia and germinating capacity during cultivation. The differences, however, gave no cause for the two strains to be cultivated separately at special temperatures.

The influence of light during the formation of conidia upon the germinating capacity had to be examined in the same manner.

Table 9: Influence of light (during the formation of the conidia) on the percentage germination of the conidia of two strains. Cultivation in Pulst's nutrient solution at room temperature.

24 hours' germination in distilled water at ~20° C. N = 20.

Cultivation in	EK 4/53		EK 7/53	
	$\bar{x}$ (ng %)	$s_{\bar{x}}$	$\bar{x}$ (ng %)	$s_{\bar{x}}$
Light	2.3	0.41	7.8	0.45
Darkness	3.1	0.38	8.0	0.55

LSD₅ = 1.27

The light had no influence upon the germinating capacity of the conidia while they were developing.

The cultures could, therefore, be stored in the laboratory at room temperature (18 to 22° C) without special lighting having to be provided. A study now had to be made to establish whether and to what extent the variations in the germination results obtained on wettable sulphur deposits when mass-spore cultures (cf. Table 5) were used, were to be attributed to differences in the degree of resistance offered by the strains contained in these cultures.

c) *The influence of several fungicides (copper oxychloride, TMTD, zinc dimethyl dithiocarbamate, wettable sulphur) on the conidial germination of the strains EK 4/53 and EK 7/53*

Schmidt (1937a), Kütke (1935) etc. have proved that a population may be a mixture of many different strains. The population percentage of the individual strains is subject to fluctuations which may be caused by the cultivation conditions (Schmidt, 1937a). It might, therefore, be conceived that variations in the effectiveness of a substance such as they occur in tests carried out at different times with mass-spore cultures, could be the outcome of a rearrangement of the percentages of spores differing in their sensitivity to this substance. In order to check this assumption, the two strains, EK 4/53 and EK 7/53, were tested for their sensitivity to copper oxychloride (Ob 21), TMTD ("Pomarsol" conc.), Zn-dimethyl dithiocarbamate ("Pomarsol" Z conc.) and wettable sulphur (particles of less than 1 μ). The effect of these substances on the germination of conidia of both strains was established in the spray test.

The efficacy of copper oxychloride (Ob 21 = "Cupravit") against conidia of the EK 4/53 and EK 7/53 strains

Ob 21 is a copper oxychloride preparation made by Farbenfabriken Bayer Aktiengesellschaft, Leverkusen, and contains 50 % Cu. Six concentrations of this preparation were first tested on each strain. In the evaluation, EK 7/53 repeatedly showed an irregular increase of ng % from approximately 15 % to approximately 80 % at concentrations of Ob 21 ranging from 0.125 to 0.25 g per litre. At a concentration of 0.5 %, no more spores germinated (cf. Fig. 12: Indicated by the pointed arrow curve). Conidia of EK 4/53 did not react in this manner. I, therefore, assumed that the regression lines would be different for the two strains. To confirm this assumption, intermediate and lower concentrations — from 0.025 g Ob 21 per litre upwards — were also tested against EK 7/53. The results are given in Fig. 12.

The conformity of the regression lines with the individual observations (K) is indicated by χ^2 (calculated) being smaller than the tabular value of χ^2 (5 %) for k-2 degrees of freedom (D. F.).

EK 4/53		EK 7/53	
χ^2 (calculated)	χ^2 (4 D. F.) (tabular value)	χ^2 (calculated)	χ^2 (8 D. F.) (tabular value)
0.5	9.5	8.1	15.5

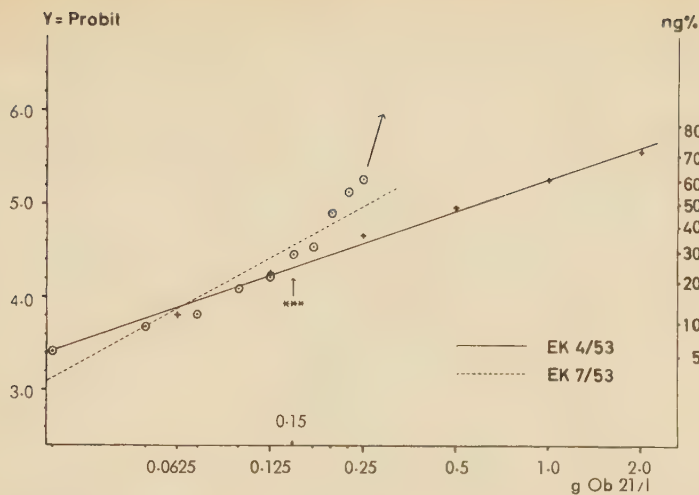


Fig. 12: Efficacy of copper oxychloride (Ob 21 = "Cupravit") against conidia of the EK 4/53 and EK 7/53 strains.
24 hours' germination at $\sim 20^{\circ}\text{C}$.

A statistical evaluation of the results according to Cochran and Cox (cf. P. 3) showed that at a concentration of even 0.15 g per litre, the difference between the reaction of the two strains is significant at $P < 0.1\%$ (cf. Table 10: $t' = 4.39$). Moreover, a theoretical Y-value of 4.32 was calculated for this concentration in respect of EK 4/53 from the equation for the regression line ($Y = 1.15352x + 2.96383$; $x = \log_2 \text{concentration} = 1.17609^1$), whereby in the calculation of t' a value of 24.9 was found for $\bar{x}$ (tr.) For s^2 , a value of 10.561 was assumed as the average of the mean squares at the concentrations of 0.125 and 0.25 g per litre.

Table 10: Efficacy of copper oxychloride (0.15 g per litre) against conidia of the EK 4/53 and EK 7/53 strains.

Concentration	EK 4/53 N=20		EK 7/53 N=10		$t' (28)$
	$\bar{x}(\text{tr.})$	$s_{\bar{x}}$	$\bar{x}(\text{tr.})$	$s_{\bar{x}}$	
0.15 g/l	24.9		29.3	0.69	4.38 ***
0.125					
0.25 g/l		0.73			

Fig. 12 shows the greater sensitivity of EK 7/53 to copper oxychloride (Ob 21) at concentrations of ≥ 0.15 g per litre.

¹⁾ To avoid logarithm < 0 , the calculation was based on g/100 litre.

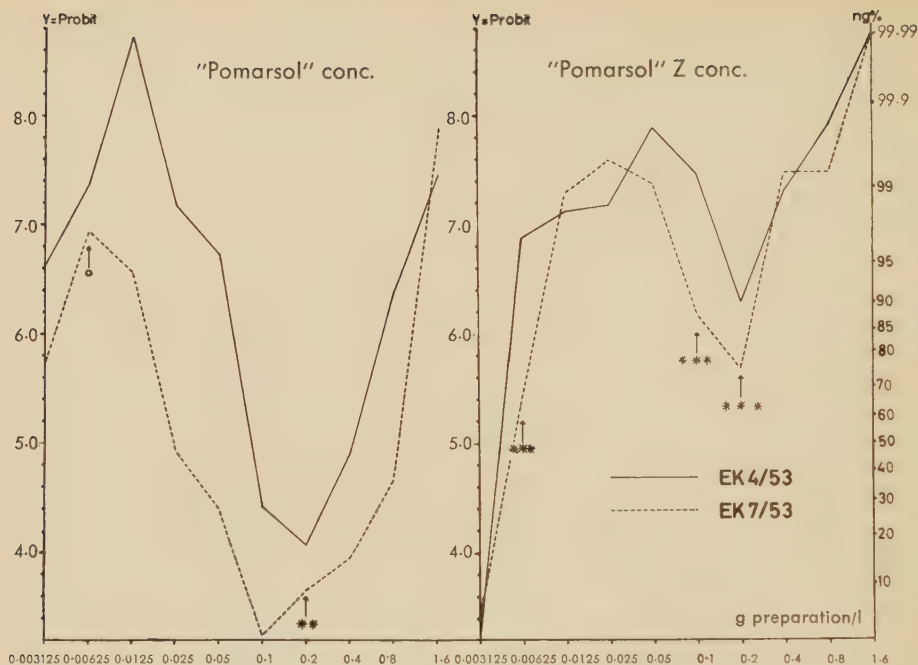


Fig. 13: Efficacy of TMTD ("Pomarsol" conc.) and Ziram ("Pomarsol" Z conc.) against conidia of the EK 4/53 and EK 7/53 strains. 24 hours' germination at 20° C.

The effectiveness of TMTD ("Pomarsol" conc.) and Ziram ("Pomarsol" Z conc.) against conidia of the EK 4/53 and EK 7/53 strains

The two preparations of Farbenfabriken Bayer Aktiengesellschaft contain 90 % Tetramethylthiuramdisulfide (TMTD) and 90 % zinc dimethyl dithiocarbamate (Ziram) respectively. They were each tested at ten different concentrations for their effectiveness. The results are given in Table 11. They could not be evaluated as a whole by means of the analysis of variance. The mean squares were so heterogeneous that it was only possible to combine small groups for calculating individual least significant differences.

It was often observed in routine tests conducted in the laboratory at the Bayer Works, that the efficacy of TMTD increased as the concentration was reduced (0.01 % to 0.00125 %).

Fig. 13 gives an explanation for this phenomenon, because this concentration range lies on a descending branch of the dosage-response curve of TMTD known since the investigations of Diamond, Horsfall, Heuberger and Stoddard (1941). Barrat and Horsfall later (1947) discussed this phenomenon in detail. Feeckes (1949) reported on similar graphs plotted for the iron and zinc salts of dimethyl dithiocarbamic acid.

The course of the curves plotted for "Pomarsol" conc. shows that strain EK 7/53 is more resistant to TMTD than strain EK 4/53, whereas the former is

Table 11: Efficacy of TMTD ("Pomarsol" conc.) and Ziram ("Pomarsol" Z conc.) against conidia of the EK 4/53 and EK 7/53 strains. 24 hours' germination at $\sim 20^{\circ}\text{C}$. N = 10.

Strain	Preparation	g preparation per litre									
		1.6	0.8	0.4	0.2	0.1	0.05	0.025	0.0125	0.00625	0.003125
EK 4/53	"Pomarsol" conc.	$\bar{x}$ (tr.)	88.0	60.7	42.7	24.5	32.0	78.8	85.7	90.0	77.8
		$s\bar{x}$	1.40	1.51	1.29	1.60	1.02	1.62	1.97	0.0	1.37
	"Pomarsol" Z conc.	$\bar{x}$ (tr.)	90.0	89.2	86.4	71.4	87.6	89.2	85.3	84.0	10.1
		$s\bar{x}$	0.0	0.86	1.58	0.58	1.30	0.86	1.71	1.43	2.20
EK 7/53	"Pomarsol" conc.	$\bar{x}$ (tr.)	89.2	37.7	22.3	17.3	10.8	31.7	43.7	78.1	61.3
		$s\bar{x}$	0.86	1.73	1.69	0.73	1.43	1.55	1.50	2.63	1.55
	"Pomarsol" Z conc.	$\bar{x}$ (tr.)	90.0	87.6	87.6	60.4	70.0	87.2	88.4	86.8	13.8
		$s\bar{x}$	0.0	1.30	1.30	1.84	1.22	1.52	1.14	1.39	1.39

more sensitive to Ob 21. The degree of significance of the difference is given in the diagram for two concentrations. The differences are significant at $P < 0.1\%$ for the other concentrations.

With respect to zinc salt, Fig. 13 shows a significant difference in the sensitivity of both strains only at three of the ten concentrations. From this test, it may be concluded that the two strains hardly differ in their reaction to Ziram.

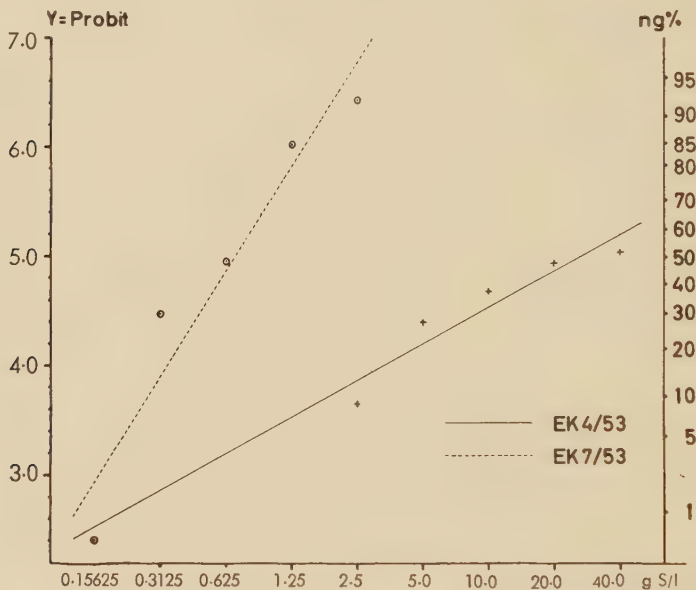


Fig. 14: Effectiveness of wettable sulphur (particles $< 1\mu$) against conidia of the EK 4/53 and EK 7/53 strains. 24 hours' germination at $\sim 20^\circ\text{C}$.

The effectiveness of wettable sulphur (particles of less than 1μ) against conidia of the EK 4/53 and EK 7/53 strains

A wettable sulphur prepared in accordance with the instructions given by Farbenfabriken Bayer Aktiengesellschaft was ground in a ball mill until all the particles had a diameter of less than 1μ . Five concentrations of this preparation were tested against each strain.

The results are plotted in Fig. 14. Since it is clear from the course of the curves that EK 7/53 is considerably more sensitive to wettable sulphur than EK 4/53, the equations for the regression lines and the least significant differences were not calculated.

The effectiveness of wettable sulphur against conidia of the EK 4/53 and EK 7/53 strains in relation to the temperature

Several reports have been published on the influence the temperature has upon the effect of sulphur. Doran (1922), Yarwood (1950) and Wilhelm

(1954) found that the effectiveness increased against several fungi when the temperature rose. In contrast, it is difficult to explain results obtained by Smith (1930a) who reported that an infection of peaches by spores of *Sclerotinia fructicola* could only be prevented at temperatures ranging from 40 to 65° F but not at temperatures of 65 to 85° F. Hamilton (1931) studied the influence of temperature upon the success of a scab control before and after infection. He found that the effectiveness of sulphur did not increase greatly during the infection period until the temperature had risen above 26° C, although he was able to prevent infections effectively at temperatures as low as 6° C. McClellan (1942) observed that the effect of sulphur was weakest at the optimum temperature for spore germination, approximately 21° C, whereas at temperatures both above and below 21° C it was found that the effect became stronger.

In the tests I carried out to examine this question, slides were prepared in the manner described earlier. Spores of EK 4/53 were treated with wettable sulphur 80 at concentrations of 2 and 4 %, while the spores of EK 7/53 were treated with 0.5 % and 0.25 % wettable sulphur because of their greater sensitivity. The slides were stored for 24 hours at temperatures of 5° C, 10° C, 15° C, 20° C and 25° C. At lower temperatures no spores germinated at all, and at higher temperatures the germination was strongly inhibited. The results are listed in Table 12, and plotted in Fig. 15.

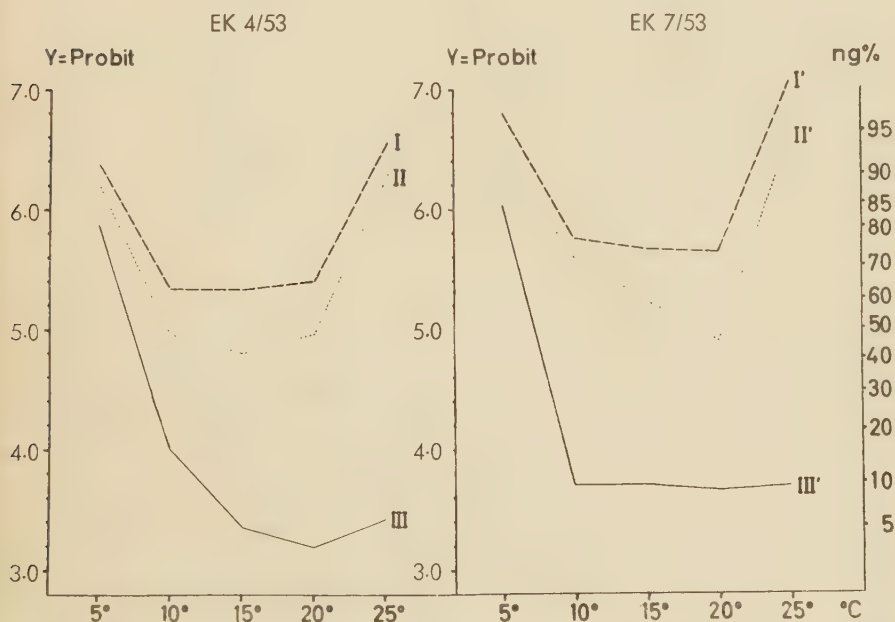


Fig. 15: Effectiveness of wettable sulphur 80 against conidia of the EK 4/53 and EK 7/53 strains at different temperatures.

24 hours' germination at ~ 20° C.

I = 4% wettable sulphur 80

II = 2% wettable sulphur 80

I' = 0.5 % wettable sulphur 80

II' = 0.25 % wettable sulphur 80

III and III' = Check

Table 12: Effectiveness of wettable sulphur 80 against conidia of the EK 4/53 and EK 7/53 strains at different temperatures. N = 5.

Treatment	EK 4/53					Treatment	EK 7/53				
	5° C	10° C	15° C	20° C	25° C		5° C	10° C	15° C	20° C	25° C
4 0/0 S	$\bar{x}$ (tr.)	73.5	52.8	52.7	54.2	76.5	80.4	61.5	59.6	59.1	82.8
	$s_{\bar{x}}$	1.06	1.18	1.46	1.24	1.75	2.65	1.35	0.56	0.57	1.91
2 0/0 S	$\bar{x}$ (tr.)	70.7	44.1	40.7	44.3	72.0	75.0	58.3	50.3	42.8	76.5
	$s_{\bar{x}}$	1.64	0.74	0.86	0.76	0.73	2.20	0.67	1.03	1.14	1.20
Check	$\bar{x}$ (tr.)	64.2	23.6	13.1	10.7	13.8	67.2	18.0	17.9	17.2	17.8
	$s_{\bar{x}}$	0.67	0.63	0.66	1.17	1.11	1.64	1.05	1.25	0.49	0.73

LSD₅ = 3.1
LSD₁ = 4.1
LSD_{0.1} = 5.3

without allowing for the value of EK 7/53, 5° C,
0.5 0/0 S and 0.25 0/0 S.

An analysis of variance for the whole test was only possible by omitting the values for EK 7/53, 5° C, 0.5 % S and 7/53, 5° C, 0.25 % S. The significance of the differences between these two and the other values was determined according to the approximate method of Cochran and Cox.

From Fig. 15, the following relations can be differentiated between the efficacy of wettable sulphur (I, I', II, II') and the germination of conidia in the checks (III, III'):

	EK 4/53		EK 7/53	
	ng ⁰ /o (III) — ng ⁰ /o (I)	ng ⁰ /o (III) — ng ⁰ /o (II)	ng ⁰ /o (III') — ng ⁰ /o (I')	ng ⁰ /o (III') — ng ⁰ /o (II')
5° C	10.8	3.0	11.6	8.0
10° C	47.4	32.8	67.6	62.8
15° C	58.0	37.4	64.8	49.6
20° C	62.2	45.2	64.8	37.4
25° C	88.4	84.6	88.6	85.0

Against strain EK 4/53, the effectiveness of the preparation increases in comparison with the check as the temperature rises, whereas against strain EK 7/53 there is an initial drop in effectiveness following a rise in temperature from 5° C to 10° C, only to increase again most noticeably at a temperature of 25° C. There is no sign of a minimum of effectiveness at the most favourable temperatures for germination, such as was established by McClellan. At the temperature optimum for the germination of conidia, ranging from 15 to 20° C, the germination is also most vigorous on treated slides (cf. Tisdale, 1925; Horsfall, 1945). In the check, the conidia of EK 7/53 germinated between 10 and 25° C more evenly yet on the whole not so well as those of EK 4/53 except at a temperature of 10° C. The much greater sensitivity of EK 7/53 to sulphur was confirmed at all temperatures whereby the similarity in curves I and I', and in curves II and II' is particularly noticeable. In view of the results obtained, it is essential for the temperature to be kept constant in the spore-germination test during the period of germination.

The effectiveness of wettable sulphur against conidia of the EK 4/53 and EK 7/53 strains on the leaf and on the slide

A preliminary experiment was carried out for the purpose of establishing whether the differences in the sensitivity of both strains to wettable sulphur determined on the slides are also apparent on the green leaf. Rich, Horsfall and Keil (1952) have explained in detail what difficulties are encountered when an attempt is made to draw conclusions from results obtained on inert material, in this case slides, as to the conditions on the living plant.

Three vaseline rings were applied to young leaves of two year old plants of the *Laxton Superb* apple variety. The leaves were secured to slides and the vaseline rings were applied as far as possible to the intercostal sections. After spraying the leaves with wettable sulphur 80 (0.5 %) by means of the pendulum sprayer, the spores were applied at the usual concentration in drops of distilled water. After 24 hours' incubation in Petri dishes at room temperature, the spore-bearing leaf parts were brightened in pyridine, dyed with cotton blue (Leben, 1952) — dissolved in lactophenol — and then the germinated conidia

were counted under the microscope in polarized aximuthal illumination. Slides treated in the usual manner were used as checks.

Accordingly, there are no significant differences between the slide test and the leaf test (cf. Blumer and Kunder t, 1950), although Marsh (1936) found that the fungicidal effect on leaves was slighter than on slides. The differences in sensitivity between EK 4/53 and EK 7/53 are significant at $P < 0.1\%$ both on the slide and on the leaf.

Table 13: Effectiveness of wettable sulphur 80 against conidia placed on leaves or, alternatively, on slides.
24 hours' germination at $\sim 20^{\circ}\text{C}$. $N = 5$.

	$\bar{x}(\text{tr.})$	$s_{\bar{x}}$	
EK 4/53 on leaf	38.08	0.911	$\text{LSD}_5 = 5.44$
EK 4/53 on slide	39.35	1.608	
EK 7/53 on leaf	50.94	1.520	
EK 7/53 on slide	53.22	2.732	

The influence of a leaf passage on the sensitivity of scab strains to wettable sulphur

In 1935, Schmidt reported that in his investigations on single-spore cultures, reisolates had the same properties as the initial forms, so that the host had not exerted any influence upon the strains. As the cultures I used had already been bred on artificial nutrient media since 1951, a check could be carried out on them to establish whether their resistance to fungitoxic substances had decreased and whether the resistance could be restored to normal by a leaf passage.

The infections were made on two year old plants of the *Laxton Superb* apple variety in the greenhouse. Contrary to the data of Wiesmann (1931), the conidia were still pathogenic despite continuous artificial cultivation. The first symptoms appeared on the plants 10 days after the infection with EK 4/53 and on the 12th day after the infection with EK 7/53. On the 18th day after the infection, the degree of infestation was about the same for both strains. The somewhat earlier appearance of the first symptoms following the infection with EK 4/53 may be due to the more rapid development of the appressoria. I also observed this in a preliminary test where drops of spore suspension were applied to a film of cellulose nitrate covering a thin layer of malt agar on a slide. With EK 4/53, the development of appressoria was observed on the first day following the application of the drops and the penetration of the germ tube through to the agar on the second day, whereas EK 7/53 took three and four days respectively for these two processes. As soon as the agar layer was reached, a vigorous mycelium growth set in.

Individual spores were recovered from the conidial strains on the leaves, and new cultures of both strains were cultivated from these. They did not differ in their habitus from those bred uninterruptedly on artificial nutrient

media. There were no variations either in the form of the conidia or in the different lengths of the germ tubes. The reaction of conidia from the reisolated and from the initial cultures to commercial wettable sulphur 80 is shown in the following table:

Table 14: Influence of a leaf passage on the reaction of conidia of the EK 4/53 and EK 7/53 strains to wettable sulphur 80.

Spray test on slides; 24 hours' germination at $\sim 20^{\circ}\text{C}$. $N = 5$.

% Wettable sulphur	Without leaf passage			With leaf passage			II—I	$t(n_R)$
	$\bar{x}$ (ng %)	$\bar{x}(\text{tr.})$ I	$s_{\bar{x}}$	$\bar{x}$ (ng %)	$\bar{x}(\text{tr.})$ II	$s_{\bar{x}}$		
EK 4/53								$t(40)$
Check	5.8	14.1	0.78	8.6	17.1	1.03	+ 3.0*	2.56
4	57.9	49.6	0.70	52.9	46.6	0.66	— 3.0*	2.53
2	46.0	42.7	1.05	33.9	35.6	0.52	— 7.1***	6.02
1	34.4	35.9	0.92	21.8	27.8	0.75	— 8.1***	6.83
0.5	28.1	32.0	0.87	18.2	25.2	0.95	— 6.8***	5.75
EK 7/53								$t(36)$
Check	15.2	22.7	1.27	9.1	17.6	0.84	— 5.1***	4.44
1	81.0	64.2	0.74	73.0	58.8	1.96	— 5.4***	11.16 ¹⁾
0.5	66.5	54.7	0.57	37.1	37.6	1.02	— 17.1***	14.77
0.25	59.3	50.4	0.72	28.8	32.3	1.04	— 18.1***	15.65
0.125	42.2	40.6	0.64	15.4	23.1	0.70	— 17.5***	15.17

¹⁾ t and P for II—I — 5.4 were calculated separately according to the simple t -test, because the high $s_{\bar{x}}$ value of 1.96 does not permit an inclusion of $\bar{x} = 58.8$ in the analysis of variance.

In the check, it was noticed that there was a definite decrease in the percentage germination of EK 4/53 conidia, with an increase in the resistance of both strains to wettable sulphur (decreasing number of non-germinated spores). It had a varying influence, however, on both strains in that the resistance of EK 7/53 to wettable sulphur became greater than that of the other strain (higher t values), whereby it should be considered that the ratio of the concentrations is the same in both cases. Consequently the observation of Schmidt (1935) could only be confirmed with respect to the morphological properties but not in respect of the reaction of the reisolated strains to wettable sulphur.

It is obvious from the investigations that the single-spore strains isolated from the population obtained in 1951 differ in their reaction to fungicides. These variations are equally apparent on slides and on leaves when wettable sulphur is used, and in fact they are also perceptible following a leaf passage on the reisolated strains. The variations in the effectiveness of wettable sulphur against conidia from several multi-spore cultures (cf. Table 5) are obviously the result of a proportional rearrangement from culture to culture among the strains differing in their degree of sensitivity to wettable sulphur. It is, therefore,

advisable to use conidia from single-spore cultures when investigating the fungitoxic properties of wettable sulphur, in order to obtain reproducible results. In my own tests, I used conidia of the sulphur-shy EK 7/53 strain in order to make a better recording of slight differences in effectiveness.

E. Studies on the fungitoxicity of wettable sulphur to conidia of the strain EK 7/53

In the examination of the fungitoxic properties of chemical substances in the slide-germination test, only the fungistatic effect is determined, in other words the effect which inhibits germination. Nothing can yet be said from the results about any fungicidal properties. In order to do so, the substance must be separated from the spores after a certain time, and the spores must then be given an opportunity to continue germinating in the most favourable medium or in distilled water. A subsequent germination of spores which at first are inhibited, is an indication of an exclusive fungistatic action. It does not appear possible to me for a clear distinction to be made between both effects because a transition from an inhibition of germination to an eradication of spores is conceivable. This eradication may take place sooner or later dependent upon the concentration of the fungitoxic substance in combination with the persistence of a given quantity of poison.

Results of scab control tests (Guilliams and Soenen, 1953; Besemer and Meyneke, 1953; Keyer and Dijksterhuis, 1953), in which wettable sulphur was applied after the occurrence of spores on the plants and yet nevertheless prevented an infection, give rise to the assumption that the sulphur is also capable of killing the spores. According to Sproston (1949), the best control results with sulphur against scab are obtained towards the end of the infection period. To ensure such success, it is essential that the preparation becomes active as soon as possible after the spores come into contact with the sulphur.

I. The initial fungicidal effect of wettable sulphur in relation to the temperature

The progress of germination following the separation of spores from the fungicide has been observed by several research workers. Liming (1932) examined the influence of evaporated sulphur on spores of *Sclerotinia cinerea* Schroet. and *Cladosporium fulvum* Cook. He placed spores in a closed cell on a glass rod and held the rod over sulphur, and then allowed the sulphur to act at different temperatures and different degrees of relative humidity. He then established in a drop of water whether the spores had retained their germinating capacity. Doran (1922) found that for sulphur to become fully effective as a fungicide, a certain temperature must be maintained over a given period. He cut a scab-infected apple into two halves, and treated one half with sulphur dust. At various intervals, he removed the spores from both halves of the apple, and allowed them to germinate separately in water. According to his results, the spores are not killed until after the fungicide has been active for

five hours at 26° C. Wilhelm (1954) found the spores of *Oidium Tuckeri* to be far more resistant to sulphur. At 16° C, some of the spores were not harmed until after the preparation had been active for one day, and more serious harm was not caused until after two to three days. Even at 30° C, 18 hours elapsed before the spores were killed.

The question of the initial fungicidal effect is of importance in connection with the adhesiveness of sulphur. When a spore is carried by rain to a leaf which has been given a prophylactic spray of sulphur, then the germination of the spore will be prevented. If the preparation is washed off to a large extent within several hours by continuous rainfall, its concentration may drop below the limit of effectiveness. A spore which is merely inhibited in its germination by the initial contact with sulphur, may germinate after all because it can retain its power to do so through the constant state of moisture on the leaf. However, if the sulphur had been strong enough to kill the spores during the few hours available, then germination would no longer be possible.

When separating sulphur from the spores, mechanical damage or any other changes apart from the harm caused by the sulphur must be avoided. This is ensured when the sulphur is separated from the spores by filtration. As the spores have an average size of $30 \times 9 \mu$, it is only necessary to use a sulphur with particles of less than 5μ in diameter. In the tests, filtration was carried out through a "Blauband 1575" type filter made by Messrs. Schleicher and Schüll, with an average pore diameter of 1.5 to 2μ .

I poured 1 ml each of a wettable sulphur suspension (0.01 %) and an EK 7/53 spore suspension (approx. 600,000 spores per ml) into small tubes 10 mm in diameter. Each batch of four tubes and a check tube containing 1 ml of water and 1 ml of spore suspension were kept at temperatures of 5°, 10°, 15°, 20°, 25° and 30° C respectively. After 3, 5, 7 and 24 hours the spores of one tube were separated from the sulphur for each temperature stage by means of filtration. This was done by placing a filter paper disk (6 cm diameter) on a glass suction filter, pouring the contents of a tube on to the disk, and then rinsing with distilled water. While the liquid was being drawn off, care had to be taken that the spores left on the filter were always under water. A brief drying of the spores resulted in a decrease of germination. The contents of

Table 15: Fungicidal effectiveness of wettable sulphur 80 (0.005 %) against conidia of the EK 7/53 strain in relation to the temperature and the persistence of sulphur.

24 hours' germination in distilled water at $\sim 20^\circ \text{C}$. N = 10.

°C	Check		3 hours		5 hours		7 hours		24 hours	
	$\bar{x}(\text{tr.})$	$s_{\bar{x}}$	$\bar{x}(\text{tr.})$	$s_{\bar{x}}$	$\bar{x}(\text{tr.})$	$s_{\bar{x}}$	$\bar{x}(\text{tr.})$	$s_{\bar{x}}$	$\bar{x}(\text{tr.})$	$s_{\bar{x}}$
5	23.7	0.91	23.4	1.05	28.0	0.85	29.3	1.28	50.9	0.82
10	26.4	1.06	24.5	0.55	33.7	2.24	37.4	0.84	74.6	1.18
15	29.8	1.35	30.2	1.28	37.3	1.20	59.6	0.87	75.9	0.78
20	28.2	1.37	37.0	1.14	56.2	0.74	69.7	1.57	90.0	0.0
25	32.5	1.61	55.0	0.89	73.2	0.78	85.1	1.98	90.0	0.0
30	39.8	1.45	84.5	2.35	90.0	0.0	90.0	0.0		

the check tube were subjected to the same filtration process after six hours. After the sulphur had been washed out, the spores were transferred to distilled water with a fine camel's hair brush. The spore suspension thus obtained was adjusted to a spore concentration of 150,000 spores per ml by centrifuging and filling up with distilled water, and applied in drops to the slide. The germination result was recorded after 24 hours of germination at approximately 20° C.

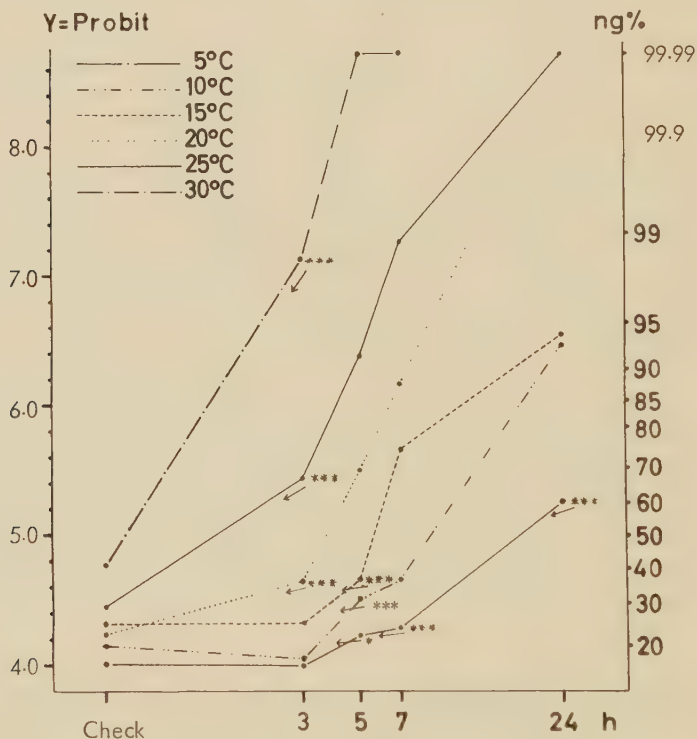


Fig. 16: Fungicidal efficacy of wettable sulphur 80 (0.005%) against conidia of the EK 7/53 strain in relation to the temperature and the persistence of sulphur. The asterisks indicate the significance of the difference of the respective value as a check.

The test was divided into groups each with homogeneous mean squares. An analysis of variance was carried out for each group. For those individual values which could not be classified in any of the groups, the significance was determined according to the t-test or, alternatively, according to the approximate method of Cochran and Cox.

It is most evident that sulphur is fungicidally effective. This also supports the assumption that sulphur which is applied as a curative spray kills the scab

spores. It was also revealed that with sulphur an observation period of 24 hours is sufficient, whereas Martin (1932) pointed out that under certain circumstances a substance does not become toxic until it has been in contact with the spores for a longer period.

Fig. 16 also shows that the initial fungicidal effect is greatly influenced by the temperature. The effect was never satisfactory at temperatures under 10° C even at the relatively high sulphur concentration of 0.005 % which corresponds to approximately 0.42 % S in the spray test. At temperatures of about 15 to 20° C, a 7 hour action of sulphur resulted in a considerably large kill of spores. The extremely rapid eradication of spores at 30° C (approx. 98 % kill after only three hours) is practically of no significance because outdoors a period of rainfall which lasts long enough for the infection to take place, is followed by a cooling off to lower temperatures (Clum, 1926; Curtis, 1935 and 1936). But even at a temperature of 25° C which must also be expected when rain falls, the fungicidal effect of sulphur is most apparent after only three hours.

II. The fungitoxic effectiveness of wettable sulphur in relation to the particle sizes of sulphur

The first indications of how important the preparation of sulphur is for its effectiveness were provided by observations on sulphur dust. As far back as 1913, Blodgett pointed out that with fine sulphur dust better results are obtained than with coarse dust because the former gives a better coverage of the treated plant parts and in fact adheres better to the plants (Hartsuijker, 1951). Wilcoxon and McCallan (1930) appear to have been the first persons who investigated the effectiveness of sulphur in relation to the particle sizes without taking into consideration the adhesiveness of the preparation. They showed in tests on conidia of *Sclerotinia americana* (Worm.) Nort. & Ezek. that ground sulphur acts better the finer it is (285 to 33 μ). According to Streeter and Rankin (1930) and Goodhue (1938), this range of particle sizes is of no interest for field applications because they experienced that particles with a diameter of more than 27 μ do not adhere to the plant. Feichtmeir (1949) tested the influence of particle sizes on effectiveness by applying four fractions of sulphur to *Sclerotinia fruticola* (Wint.) Rehm and *Erysiphe graminis hordei* D.C. He found that the degree of effectiveness increased as the size of the particles was reduced from 15—16 μ to 1—2 μ . The coarse particles were relatively more effective against *Erysiphe* than against *Sclerotinia*. Other research workers, too, have found that sulphur is more effective when the particles are reduced in size (Hamilton, Palmiter and Mack, 1943; Hartsuijker, 1951; McNew, 1953 etc.). This was also established for copper fungicides (Heuberger and Horsfall, 1939). The increase in effectiveness proportional to reductions in the particle size was found not only in spore-germination tests in the laboratory but also in outdoor scab tests (Soenen, 1952; Wenzl, 1952; Lorenz, 1953).

McNew (1952), however, observed that particles of 6 to 10 μ in diameter had a definite optimal effect when Phygon (2,3-dichlor-1,4-naphthoquinone) was used. He divided the sizes of particles ranging from 1 μ to 40 μ diameter into five fractions, and also tested the effectiveness of particles with a diameter

of more than 40 μ . Too many particles of 1 to 6 μ diameter were required to kill a spore whereas too many particles with a diameter of more than 10 μ had to be applied to guarantee that at least one particle would reach a spore. This raised the question as to whether the effect of a fungitoxic substance decreases again below a definite particle size. Mc New is of the opinion that the optimum must be fixed separately for each individual preparation.

The observations of Feichtmeir (1949) and Loewel (1952) that coarse wettable sulphur is more effective against powdery mildew than a fine product would suggest that it is inadvisable to prepare a wettable sulphur which is too fine. The fact that the vaporization and oxidation of sulphur (Lilly and Barnett, 1951, P. 251—256), and consequently the danger of burns on green plants (Turrell, 1950 etc.) increase when the particle sizes are reduced, also supports the advisability of not making the particles too fine. I carried out a number of tests with *Venturia inaequalis* to obtain a decision on this question.

In order to assess the effectiveness of wettable sulphur of different particle sizes, any influence of the auxiliaries must be eliminated. If the influence of the particle size on the effectiveness of sulphur is to be examined on its own, then the fractions required for this must be obtained from one particular make of wettable sulphur so that the fractions contain the same inert material. The separation of the groups of particle sizes is best done by sedimentation. From a commercial wettable sulphur 80, I obtained the fractions 3—8 μ , 2—6 μ , 1—4 μ and < 2 μ diameter by sedimentation in 1,000 ml measuring flasks. The fractions < 1 μ and < 0.5 μ were obtained by firstly grinding the wettable sulphur in a ball mill to < 1 μ and then by sedimentation in a centrifuge, to separate the two fractions. The particle spectra of the individual fractions (1—4) were determined by an analysis of sedimentation.

The wettable sulphur fractions were tested for their effectiveness against conidia of the EK 7/53 strain in six concentrations and four replications in the spray test.

The results have been evaluated according to the method of Finney (1952). The regression equations given in Table 17 are calculated from 24 means

Table 16: Particle spectra of four fractions of a commercial wettable sulphur 80. Percent by weight.

Fraction	Particle diameter in μ							
	< 1	1—2	2—3	3—4	4—6	6—8	8—10	> 10
3—8 μ	3.07	4.13	14.90	18.80	27.40	16.40	9.91	5.42
2—6 μ	3.04	12.40	62.60		20.90	1.03		—
1—4 μ	10.15	31.20	37.80	12.05	3.09	2.15	1.95	1.70
< 2 μ	56.35	41.85	1.80	—		—	—	—

of each 10 individual values. Table 17a has been drawn up from the m_{11}/m_0 and b_{11}/b_0 columns of Table 17. Table 17a shows the significance of the differences between the log-LD₅₀ values (m) and the regression coefficients (b) of the individual fractions.

The LD₅₀ becomes smaller as the particle size decreases (cf. Table 17). This decrease is significant at $P < 0.1\%$ up to a particle size of $2\ \mu$, whereas the differences of the LD₅₀ for particles $< 2\ \mu$ are not significant. Fig. 12 shows that

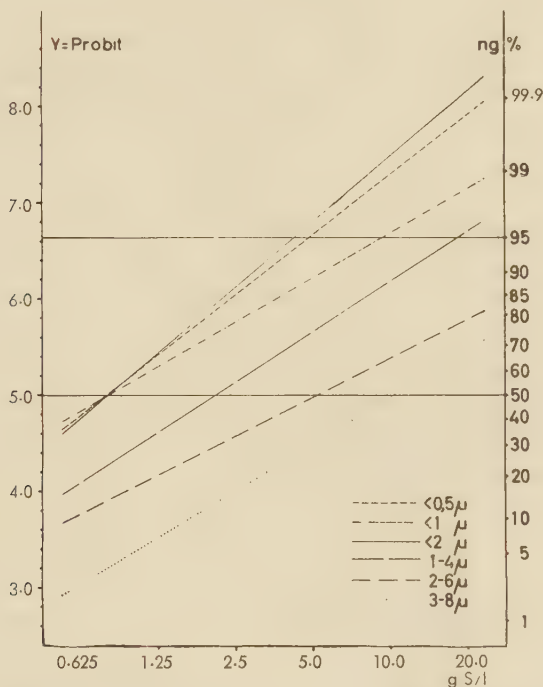


Fig. 17: Effectiveness of wettable sulphur of different particle sizes against conidia of the EK 7/53 strain.
24 hours' germination at $\sim 20^\circ\text{C}$.

with the fractions $< 2\ \mu$, $< 1\ \mu$ and $< 0.5\ \mu$ there is a variation in the grading of effectiveness at higher concentrations, in that the LD₉₅ values of the fractions $< 2\ \mu$ and $< 0.5\ \mu$ do not differ significantly whereas the LD₉₅ of the fraction $< 1\ \mu$, significant at $P < 0.1\%$, is higher. This lower effectiveness of particles with an average diameter which is between that of the other fractions ($< 2\ \mu$ and $< 0.5\ \mu$), is not explicable biologically.

Accordingly, the effectiveness of sulphur increases at first as the particle size is reduced, and reaches a maximum at the particle spectrum given in Table 16 for the fraction $< 2\ \mu$. This maximum is at least not exceeded when the particle size is further reduced.

Table 17: Effectiveness of wettable sulphur of different particle sizes against conidia of the EK 7/53 strain. Probit analysis.

Fraction	$Y = bx + (\bar{y} - b\bar{x})$	$\chi^2_{(22)}$	m	s_m	$m \pm t \cdot s_m$ m_u m_o	LD 50 (10^{-1} g/l)	LD 95 (10^{-1} g/l)	b	s_b	$b \pm t \cdot s_b$ b_u b_o	P%
3—8 μ	$Y = 1.6146 x + 1.7445$	26.0	2.0163	0.0156	1.9857 2.0469 1.9761 2.0565 1.9650 2.0676	103.80 ± 3.720		1.6146	0.0670	1.4833 1.7459 1.4420 1.7872 1.3941 1.8351	5 1 0.1
2—6 μ	$Y = 1.3407 x + 2.6990$	38.4	1.7163	0.0199	1.6750 1.7576 1.6601 1.7725 1.6407 1.7919	52.04 ± 1.199		1.3407	0.0584	1.2196 1.4618 1.1761 1.5053 1.1192 1.5622	5 1 0.1
1—4 μ	$Y = 1.7333 x + 2.7091$	37.3	1.3480	0.0217	1.3030 1.3930 1.2868 1.4092 1.2656 1.4304	22.29 ± 1.168		1.7333	0.0600	1.6089 1.8577 1.5642 1.9024 1.5053 1.9608	5 1 0.1
< 2 μ	$Y = 2.2593 x + 2.9585$	51.0	0.9036	0.0208	0.8605 0.9467 0.8450 0.9622 0.8247 0.9825	8.01 ± 1.105	42.82 ± 5.289	2.2593	0.1021	2.0475 2.4711 1.9715 2.5471 1.8721 2.6465	5 1 0.1
< 1 μ	$Y = 1.5299 x + 3.6192$	41.5	0.9025	0.0267	0.8471 0.9579 0.8272 0.9778 0.8013 1.0037	7.99 ± 1.136	95.00 ± 15.579	1.5299	0.0669	1.3911 1.6687 1.3413 1.7185 1.2762 1.7836	5 1 0.1
< 0.5 μ	$Y = 2.0625 x + 3.1594$	42.4	0.8924	0.0209	0.8491 0.9357 0.8335 0.9513 0.8131 0.9717	7.81 ± 1.104	48.97 ± 5.891	2.0625	0.0858	1.8846 2.2404 1.8206 2.3044 1.7371 2.3879	5 1 0.1

Table values of $\chi^2_{(22)}$ 5% = 33.9

1% = 40.3

0.1% = 46.3

χ^2 = factor for assessing the agreement of the degrees of regression with the different observations (P. 55)*.

In the computation of the confidence limits, $t(\infty)$ was included for the non-significant χ^2 , and the factor of heterogeneity and $t_{(22)}$ for the significant χ^2 (P. 64).

$Y = \bar{y} + b(x - \bar{x}) = bx + (\bar{y} - b\bar{x})$ = equations for the degrees of regression in Fig. 17 (P. 61).

$m = \log LD 50$ (P. 55); s_m = standard deviation of m (P. 35); m_u and m_o = lower and upper confidence limit of m (P. 60).

b = Coefficient of regression (P. 53); s_b = standard deviation of b (P. 54); b_u and b_o = lower and upper confidence limit of b (S. 243)

* The page numbers quoted in this table refer to the paper of Finney (1952).

Table 17a: Statistical significance of the differences between m and b of the individual fractions (compiled from Table 17).

m \ b	3—8 μ	2—6 μ	1—4 μ	< 2 μ	< 1 μ	< 0.5 μ
3—8 μ		1	0	3	0	3
2—6 μ	3		2	3	0	3
1—4 μ	3	3		2	0	1
< 2 μ	3	3	3		3	0
< 1 μ	3	3	3	0		2
< 0.5 μ	3	3	3	0	0	

0 = not significant

1 = significant at $P < 5\%$

2 = significant at $P < 1\%$

3 = significant at $P < 0,1\%$

III. The persistence of wettable sulphur in relation to the weather factors and the particle sizes of sulphur

The success of a prophylactic scab control with wettable sulphur depends largely upon whether, and if so how long, the sulphur remains sufficiently active. There are several conceivable causes which may be responsible for a drop in its fungitoxic efficacy before scab spores reach the sprayed leaf. Atmospheric influences could cause a change in the molecular structure of sulphur and thus affect its reactivity (De Ong, 1924; Feichtmeir, 1949). A reaction with those auxiliaries added to each wettable sulphur (wetting agents, stickers etc.), which change the physical properties, could be stimulated by substances in the atmosphere. A formation of conglomerates, for example, leads to a change in the surface conditions which is mostly accompanied by a reduction of efficacy and adhesiveness (Feichtmeir, 1949). Curtis (1943 and 1944) and Rich (1954) presume that the host plant causes a change in the sulphur due to leaf excretions.

Doran (1922) observed that a spray coating of lime-sulphur mixture which dried on slowly was by no means as effective as a coating which dried on rapidly, because slow drying results in a complete decomposition of the sul-

phides present. Moreover, the moisture is alleged to assist an oxidative decomposition.

The drop in the fungitoxic efficacy may be due to a loss of sulphur resulting from vaporization. We know that sulphur vaporizes at high temperatures (Tucker, 1929; Liming, 1932; Fouretier and Boullé, 1946a and b, Turrell, 1947), whereby the rate of vaporization increases continuously as the temperature goes up. At temperatures of 24–26 °C, 30–35 °C and 40–45 °C, Tucker found the ratio of sublimation to be 1:6:80. The temperatures at which the degree of vaporization of elementary sulphur was examined, were mostly too high to be of any interest to us in the biological question with which we are concerned (Tucker: 25–50 °; Liming: 24–93 °; Fouretier: 35–50 °C). Goodwin and Martin (1928) reported that humidity and light do not influence the vaporization of sulphur. Tucker (1929) confirmed this observation, and was in fact unable to establish any cases of particle sizes having an influence on vaporization. According to Frear (1948, P. 244), however, fine crystalline sulphur vaporizes more rapidly than coarse amorphous particles of sulphur. Fouretier and Boullé (1946) tested the vaporization of sulphur in a dry atmosphere and in an atmosphere saturated with water vapour but found no difference.

The investigations are more or less of a purely physical and chemical nature while on the other hand a biological test has never yet been made of the fungicidal effect of sulphur residue left over as a coating after vaporization or oxidation. This residual effect, however, is most important for the success of a prophylactic application of sulphur.

1. Method

The persistence test was carried out on slides in the laboratory. One reason for doing the test in the laboratory was that by using the pendulum sprayer an equal quantity of sulphur per unit of area could be applied to each slide which was essential for the success of the test. Conidia of the EK 753 strain were used. To eliminate as far as possible any variations in the germinating power of the spores, a strict procedure was followed in that only spores of one culture from the same culture vessel were used for each test. The slides were sprayed with wettable sulphur by means of the pendulum sprayer, and then exposed to constant atmospheric conditions. After a definite exposure time had elapsed, more slides were sprayed with the same quantity of sulphur. In this manner, it was possible to apply spores of one and the same culture simultaneously to freshly sprayed slides and to those which had been exposed to atmospheric influences for a certain period. Provided an equal quantity of sulphur was sprayed on each slide, then any differences in the effect could only have been due to atmospheric influences. By determining the percentage of non-germinated conidia on the differently treated slides, the persistence could be recorded numerically.

The particle size of the sulphur was varied in order to establish its influence on the fungitoxic persistence of the product. The concentration of the spray liquid was adjusted so that about 50 % of the conidia germinated on freshly sprayed slides (0.25 % wettable sulphur 80 or, alternatively, 0.125 % S for the fractions).

A special point was made of keeping the atmospheric conditions constant. On the one hand I had to reckon with variations in the percentages of certain gases in the air (e. g. H_2S , SO_2) due to the chemical factory being so near with its discharge of waste gases. On the other hand, the atmosphere in a closed room may be enriched with sulphur vapours or gases due to vaporization, oxidation or reduction of the sulphur, whereby an increased saturation reduces further vaporization or formation of gas (Beyer, 1951). In nature, however, all vapours or gaseous decomposition products can escape immediately due to the constant circulation of air. Vaporization or conversion to volatile gases may, therefore, be stronger under natural conditions than in closed rooms. I, therefore, placed the slides in a closed system with a constant, weak circulation of air (measurable), in which the air flowing through was cleared of gaseous impurities. Gauges were fitted to measure and regulate the temperature, relative humidity and intensity of exposure.

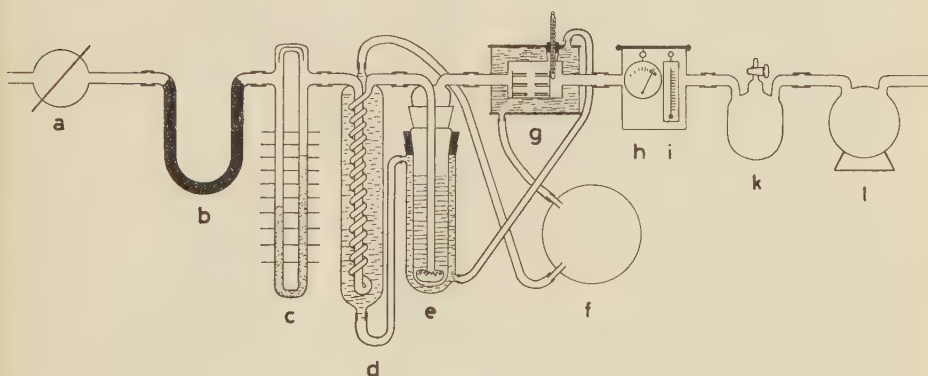


Fig. 18: Apparatus for testing the persistence of fungicides in the laboratory.

a = gas meter; b = U-tube containing silica gel; c = Riesenfeld's flowmeter; d = condenser; e = frit wash bottle; f = Höppler type thermostat; g = vessel for holding the slides; h = hair hygograph; i = thermometer; k = three-necked flask; l = vacuum pump.

It was also intended to use the apparatus for giving a quantitative analysis of the escaping sulphur vapours and gases. Due to the blank values being too high, however, it was not possible to obtain satisfactory results with several analytical methods.

I made use of the "Wisa" type diaphragm pump (Fig. 18, l) made by Messrs. Brandt of Bochum-Dahlhausen, for producing a constant air stream. As the air stream was only intended to remove any vapours or gaseous decomposition products of sulphur from the vessel containing the slides, a velocity of flow amounting to approximately eight litres of air per hour proved to be adequate. Because the pumping speed and the pressure capacity of the pump were too high, I reduced the velocity of flow by dividing the air stream when it was drawn off. The air passed from the system into a three-necked flask (k). Air from outside was able to enter this flask at the same time through a glass cock by

means of which the velocity of flow in the system could be regulated as required. The wider the cock was opened, the less was the quantity of air which passed through the system, and vice-versa. The velocity of flow was read off on a Riesenfeld's flowmeter (c). The total quantity of air flowing through the system was measured in a "Metron" type gas meter (a) (made by Messrs. Gustav Griesel K. G., Essen) which was located at the end of the system. The air was cleared of coarse dirt particles in the water of the gas meter, while the gaseous impurities were removed in silica gel (b). In order to raise the temperature of the air to any required degree between 15°C and $50^{\circ} \pm 0.05^{\circ}\text{C}$, it was subsequently

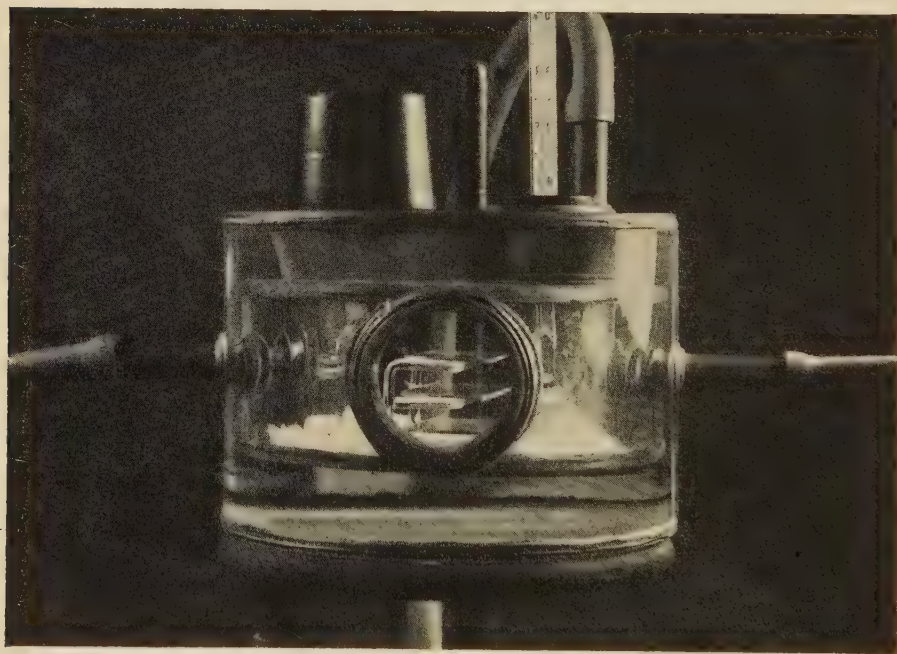


Fig. 19: Vessel for holding the slides in the persistence test.

passed through a "Dimrod" type condenser (d). The jacket of the condenser was connected to a "Höppler" type thermostat (f). To regulate the relative humidity, the air then flowed into a frit wash bottle (e) which was filled either with water to give the highest possible relative humidity — 85 to 95 % dependent upon the temperature — or with phosphorus pentoxide in phosphoric acid to dry the air to 15 % relative humidity. From the wash bottle, the air then passed into the plexiglass vessel (g) designed for holding the slides (Fig. 19).

The slides were inserted through the side opening (35 mm diameter) into the interior of the vessel (g), and located on small glass stands which were large

enough to take six slides. The vessel was surrounded by a water jacket to regulate the temperature. A thermometer was inserted for measuring the temperature, and made air-tight. The relative humidity in this vessel could not be measured because the opening was too small to insert a hygrograph. Therefore, it was measured in an adjoining plexiglass vessel (h) by means of a hair hygrograph. The absolute humidity in vessel (h) was calculated from the relative humidity and the temperature. When the calculated absolute humidity was based on the temperature measured in vessel (g), the relative humidity was obtained for the same vessel (g).

The entire apparatus was set up in a small compartment in the laboratory. Five neon tubes were fitted to the ceiling about 70 cm above the vessel (g). The light intensity in vessel (g) was measured by means of a light meter. As the photocell belonging to the meter could not be located in the vessel, a mean of 1220 lux was calculated for the centre of the vessel (g) from the values measured above and below it. Comparison tests were carried out with the compartment completely blacked out.

2. Results of the investigations

To begin with, a study was made of the influence of temperature, light and relative humidity on the persistence of sulphur. The results were evaluated by means of an analysis of variance. The temperature was tested at 15 °C, 20 °C, 25 °C and 30 °C $\pm$ 0.05 °C at a light intensity of 1220 lux and in complete darkness. The results are given in Table 18.

Table 18: Influence of temperature and light on the persistence of wettable sulphur 80 (0.25 %) with a 24 hour exposure of the spray coatings in constant relative humidity (85 to 95 %).

Test carried out on conidia of the EK 7/53 strain. 24 hours' germination at $\sim$ 20 °C. N = 10.

	not exposed			exposed			I - II
	$\bar{x}$ (ng ⁰ /o)	$\bar{x}$ (tr.) I	$s_{\bar{x}}$	$\bar{x}$ (ng ⁰ /o)	$\bar{x}$ (tr.) II	$s_{\bar{x}}$	
light							
15 °C	55.9	48.40	0.819	43.6	41.33	0.602	7.07
20 °C	54.3	47.48	0.765	34.9	36.23	0.472	11.25
25 °C	59.7	50.60	0.960	33.8	35.55	0.718	15.05
30 °C	56.5	48.75	1.118	13.1	20.99	1.199	27.76
dark							
15 °C	54.6	47.65	0.720	42.7	40.79	0.488	6.86
20 °C	54.1	47.37	0.718	34.4	35.92	0.566	11.45
25 °C	57.9	49.57	0.919	33.0	35.06	0.707	14.51
30 °C	51.6	45.93	0.949	11.8	19.96	0.840	25.97

LSD_s = 2.15

LSD_I = 2.84

LSD_{0.1} = 3.65

Table 18a: Analysis of variance to Table 18:

	n	SQ	s ²	F	F (corrected)
Total	159	14,715.14			
Temperature (T.)	3	2,639.33	879.78	150.13 ***	152.74 ***
Light (L.)	1	31.33	31.33	5.35 *	5.44 *
Treatment (Trea.)	1	8,988.00	8,988.00	1,533.79 ***	1,560.42 ***
T. × L.	3	16.10	5.37	0.92 °	
T. × Trea.	3	2,187.10	729.03	124.41 ***	126.57 ***
L. × Trea.	1	3.43	3.43	0.59 °	
T. × L. × Trea.	3	5.52	1.84	0.31 °	
Remainder	144	844.33	5.86		

The insignificant interactions T × L, L × Trea. and T × L × Trea. are combined with SQ (discrepancy) = 844.33 to determine a new remainder.

Table 18b: Determination of s² remainder (corrected).

	n	SQ	s ²
T. × L.	3	16.10	
L. × Trea.	1	3.43	
T. × L. × Trea.	3	5.52	
Remainder	144	844.33	
Remainder (corrected)	151	869.38	5.76

The gradient of the differences between the values for exposed and unexposed sulphur coatings (cf. Table 18) shows that the 24 hour airing of the sulphur in light and darkness resulted in a loss of efficacy which increased as the temperature rose. The light can be ignored as a factor governing the loss of efficacy of sulphur because at equal temperatures there are no significant differences between light and darkness.

The relation of persistence to relative humidity was tested at 15 % and 85 % relative humidity and at 25 °C and 30 °C.

Table 19: Influence of relative humidity on the effectiveness of wettable sulphur 80 (0.25 %) with a 24 hour exposure of the spray coatings on light (1220 lux) at 25 °C and 30 °C.

Test carried out on conidia of the EK 7/53 strain. 24 hours' germination at $\sim 20^{\circ}\text{C}$. N = 10.

	not exposed			exposed			I—II
	$\bar{x}$	$\bar{x}$ (tr.)	$s_{\bar{x}}$	$\bar{x}$	$\bar{x}$ (tr.)	$s_{\bar{x}}$	
	(ng ^o /o)	I		(ng ^o /o)	II		
25° C							
15% R. H.	61.8	51.86	1.089	62.8	52.46	0.950	— 0.60
85% R. H.	59.7	50.60	0.960	33.8	35.55	0.718	15.05
30° C							
15% R. H.	59.6	50.56	1.168	49.8	44.90	0.792	5.66
85% R. H.	56.5	48.75	1.118	13.1	20.99	1.199	27.76

LSD₅ = 2.71

LSD₁ = 3.60

LSD_{0.1} = 4.70

Table 19a: Analysis of variance to Table 19

	n	SQ	s ²	F
Total	79	9,089.75		
Temperature (T.)	1	798.21	798.21	86.45 ***
Relative humidity (R. H.)	1	2,407.91	2,407.91	260.79 ***
Treatment (Trea.)	1	2,864.42	2,864.42	310.23 ***
T. × R. H.	1	71.26	71.26	7.72 **
T. × Trea.	1	449.83	449.83	48.72 ***
R. H. × Trea.	1	1,781.33	1,781.33	192.93 ***
T. × R. H. × Trea.	1	52.00	52.00	5.63 *
Remainder	72	664.79	9.23	

The persistence of sulphur depends largely upon the relative humidity. At 25 °C the efficacy decreased only in humid air, and even at 30 °C the loss of efficacy in dry air is only slight in proportion to that in moist air.

The influence of the exposure time on the loss of efficacy was examined on four fractions of sulphur. At the same time the influence of the particle size on persistence was also determined.

From the figures given in Table 20, it is obvious that the loss of efficacy (I—II) increases for all fractions as the exposure time is lengthened. This increase is all the greater the smaller the particles are, whereby the efficacy losses for a 12 hour exposure must be equally set at 1.00 because the tests were carried out at different times. The efficacy loss of sulphur coatings exposed for

12 hours is slightest for the smallest particles because after such a short exposure the better effectiveness of fine particles is still strongly evident in the biological test. However, as the time of exposure was lengthened, this higher degree of efficacy no longer counterbalanced the increasingly heavy loss of substance with finer particles. This explains the tenfold increase in the loss of efficacy during the relatively short period of 60 hours for the fraction $< 1 \mu$ in comparison with the twofold increase for the fraction 2—6 μ .

Table 20: Influence of the exposure time and the particle size of sulphur on the efficacy of wettable sulphur (0.125 %) when the spray coatings are exposed in light (1220 lux) at 30° and 85 % relative humidity.

Test carried out on conidia of the EK 7/53 strain. 24 hours' germination at $\sim 20^\circ \text{C}$. N = 10.

Exposure time expressed in hours	not exposed			exposed			I—II	I—II 12 h = 1.00
	$\bar{x}$	$\bar{x}(\text{tr.})$	$s_{\bar{x}}$	$\bar{x}$	$\bar{x}(\text{tr.})$	$s_{\bar{x}}$		
	(ng 0/0)	I		(ng 0/0)	II			
<u>2—6 μ</u>								
12 h	25.6	30.37	0.690	16.2	23.64	0.842	6.73	1.00
24 h	25.5	30.27	0.974	14.0	21.82	1.055	8.45	1.25
36 h	23.5	28.92	0.892	11.9	20.00	1.072	8.92	1.32
48 h	21.9	27.85	0.749	8.7	17.05	0.751	10.80	1.60
60 h	20.9	27.15	0.785	6.2	14.20	0.898	12.95	1.92
<u>1—4 μ</u>								
12 h	38.0	38.07	0.640	26.0	30.59	0.889	7.48	1.00
24 h	37.8	37.93	0.865	21.8	27.76	0.967	10.17	1.36
36 h	36.1	36.91	0.946	16.6	23.90	1.089	13.01	1.74
48 h	40.6	39.60	0.758	13.9	21.82	0.791	17.78	2.38
60 h	43.0	40.99	0.707	9.3	17.67	0.718	23.23	3.11
<u>< 2 μ</u>								
12 h	75.4	60.35	1.001	69.0	56.21	0.725	4.14	1.00
24 h	74.0	59.38	0.706	63.6	52.90	0.562	6.48	1.56
36 h	65.2	53.89	0.803	44.8	42.02	0.637	11.48	2.77
48 h	75.0	60.09	0.919	46.4	42.94	0.675	17.15	4.14
60 h	76.4	60.99	0.755	21.0	27.24	0.638	33.75	8.15
<u>< 1 μ</u>								
12 h	72.3	58.29	0.732	66.9	54.91	0.623	3.38	1.00
24 h	71.4	57.70	0.601	55.8	48.33	0.683	9.37	2.77
36 h	62.4	52.21	0.812	40.0	39.22	0.859	12.99	3.84
48 h	74.4	59.64	0.640	45.4	42.36	0.702	17.28	5.12
60 h	75.8	60.62	0.951	18.6	25.48	0.869	35.14	10.41

LSD₅ = 2.15

LSD₁ = 2.82

LSD_{0.1} = 3.62

Table 20 a: Analysis of variance to Table 20:

	n	SQ	s ²	F
Total	399	89,002.73		
Exposure time (T.)	4	4,788.84	1,197.21	201.44 ***
Particle sizes (P.)	3	55,385.12	18,461.71	3,106.39 ***
Treatment (Trea.)	1	18,381.94	18,381.94	3,092.97 ***
T. × P.	12	1,169.91	97.49	16.40 ***
T. × Trea.	4	5,178.83	1,294.71	217.85 ***
P. × Trea.	3	552.26	184.09	30.98 ***
T. × P. × Trea.	12	1,405.90	117.16	19.71 ***
Remainder	360	2,139.93	5.94	

From the investigations it is evident that a wettable sulphur with particles of less than 1 μ diameter gives a good effect when applied at short notice but it is also clear that in view of the short persistence of such a fine wettable sulphur it is inexpedient to use it for protective scab control.

IV. Tenacity of wettable sulphur deposits in relation to the particle size of the sulphur

The tenacity of fungicide deposits is determined by the intensity of the rain (Girard, 1892; Horsfall, 1945), the strength of the fungicide deposit (Young and Tisdale, 1929), the nature of the surface (Miller, 1943; Blumer and Kundert, 1950; Ehlers, 1953b) and the type of additives used (Guillon and Gouirand, 1898; White, 1936; Garman, 1950). Mostly it is tested by means of chemical, gravimetric or optical methods. Results obtained from a test of this description, however, do not permit any definite statements to be made about the biological effect of the residual deposit (Heuberger, 1940; Miller, 1943). For example, the coarse particles of a wettable sulphur deposit which do not adhere so effectively are washed off first whereby the residual sulphur consists of finer particles. This process cannot be proved on the photographic plate, by weighing or in the chemical analysis. It is also probable that hydrophile rather than hydrophobe sulphur forms will be washed out, with the greater likelihood of amorphous sulphur being dissolved out of a deposit by rain. The importance of such selection processes which are not recognizable with the above-mentioned methods, will be understood when it is considered that fine particles are more fungicidal and that hydrophobe and crystalline forms of sulphur are less fungicidal. Elemental sulphur which is capable of penetrating into the leaf (Turrell, 1950; Marsh, 1951) is also covered by the chemical analysis without it being certain whether it can exert a fungitoxic effect at all. Therefore, when we speak of tenacity in this paper, we shall understand it to mean the preservation of the fungitoxic efficacy during rainfall because after all it is only this which is of practical importance.

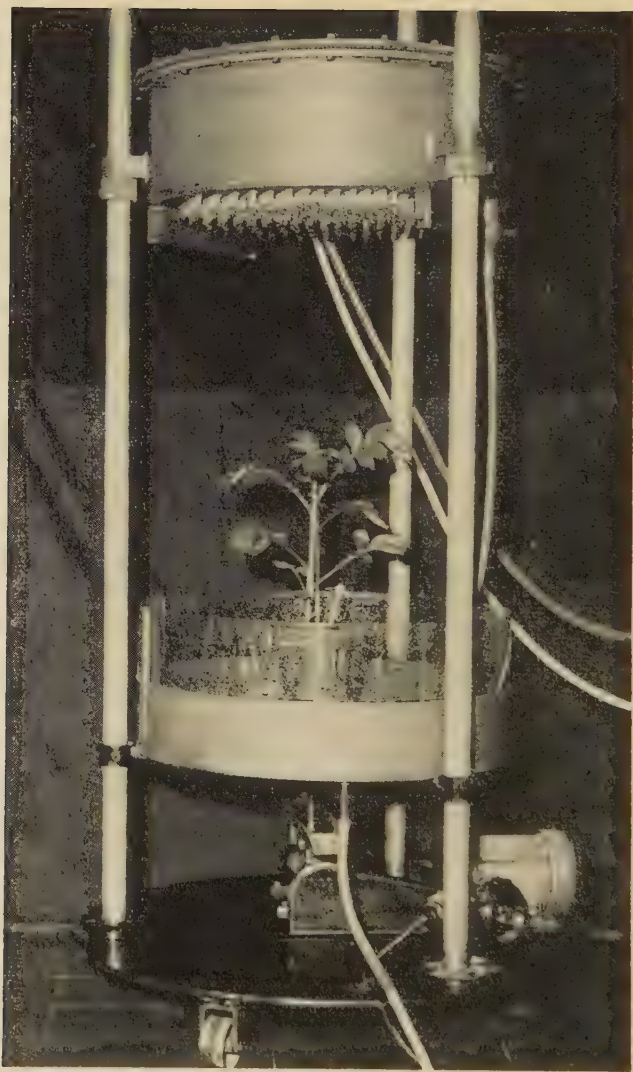


Fig. 20: Rain-exposure-test apparatus; full view.

The tenacity is tested either by means of the washing-off process or by the artificial rain method. In the washing-off process, slides or leaves are moved about in water (Montgomery and Moore, 1938; Heuberger, 1940) or else immersed in water which is caused to circulate by an agitator (Blumer and Kundert, 1950). The exposure to artificial rain of sprayed slides or leaves gives a better reproduction of natural conditions.

Zäch described an apparatus for producing artificial rain in 1947. The individual drops are produced by splitting up a jet of water on a slanting plate. The even distribution of the rain on the surface to be wetted depends upon the force of the water jet. If the force of the jet is varied, the size of the drops and the amount of rain (relatively large) will also be varied.

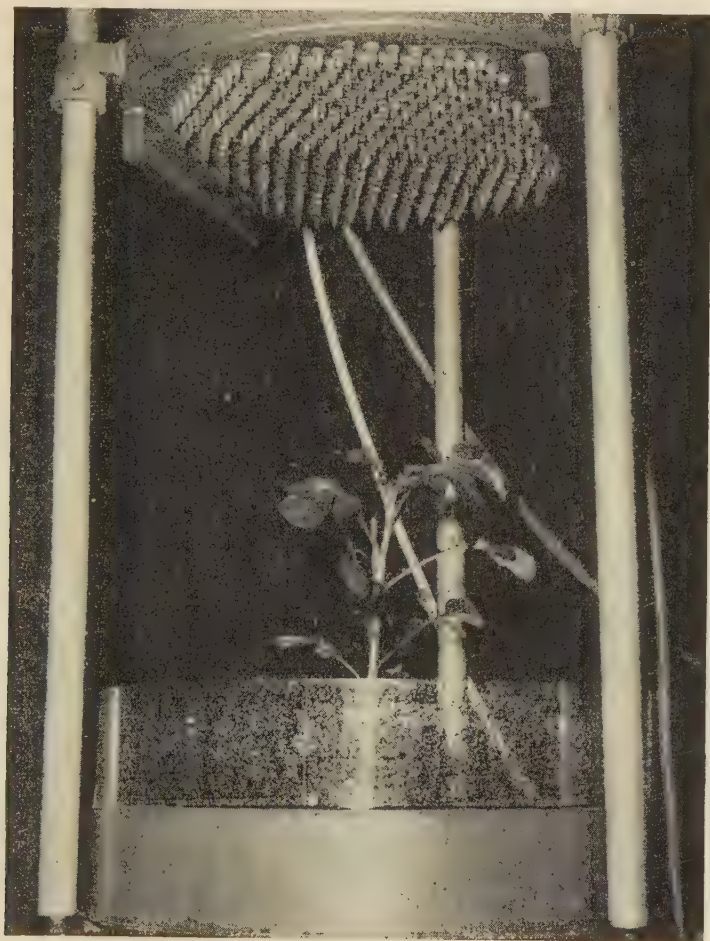


Fig. 21: Artificial rain apparatus being used for wetting a plant.

1. Method

I developed my own apparatus (Fig. 20 and 21) for carrying out my investigations and also for later use at the Biological Institute of Farbenfabriken Bayer Aktiengesellschaft. Distilled water flows out of a closed container through capillary tubes under a given pressure. Each capillary tube contains a nichrome

wire with a ring at its bottom end. The water collects in the ring until a drop breaks away. The size of the drop depends upon the diameter of the ring. The drop velocity may be influenced by the pressure bearing on the vessel, and varied within certain limits.

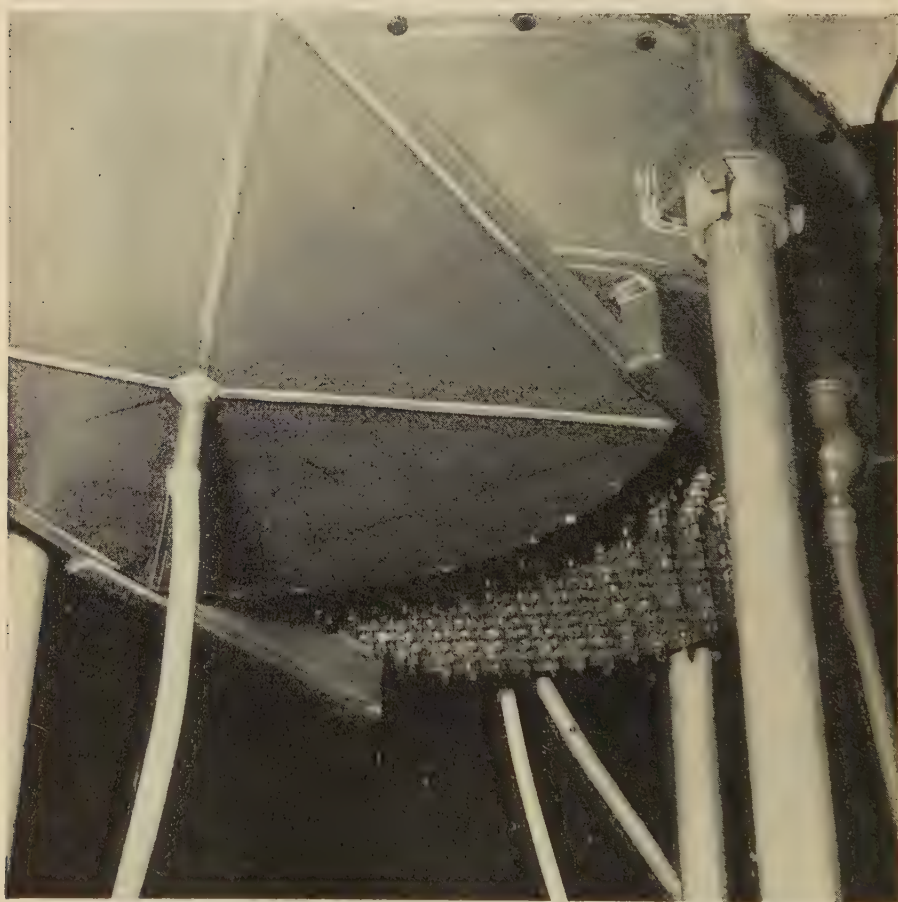


Fig. 22: Upper section of the artificial rain apparatus with slide.

At a pressure of 40 mm Hg, 1 mm of rain falls to the minute. The exposure time can be limited by manipulating a slide (Fig. 22). The object to be wetted can be moved backwards and forwards on a trolley underneath the surface covered with the capillary tubes (Fig. 24). The trolley which is driven by a motor via a gear unit, can cover a distance of 15 cm to the minute. The special

arrangement of the capillaries to conform with the direction in which the trolley moves (Fig. 23) enables every point of the leaves or slides to be hit by raindrops. The height from which the raindrops fall can be varied within certain limits.



Fig. 23: Top section of the artificial rain apparatus fitted with capillaries.

2. Results of the investigations

The fractions $2-6\ \mu$, $1-4\ \mu$, $< 2\ \mu$ and $< 1\ \mu$ were sprayed on slides at 1 %. The slides were not specially prepared. The spray deposit was able to dry on at room temperature before being exposed to rain. Afterwards, the rain water still adhering to the slides was able to evaporate into the air. The slides were then left to stand in Petri dishes for 24 hours at approximately 20°C , that is

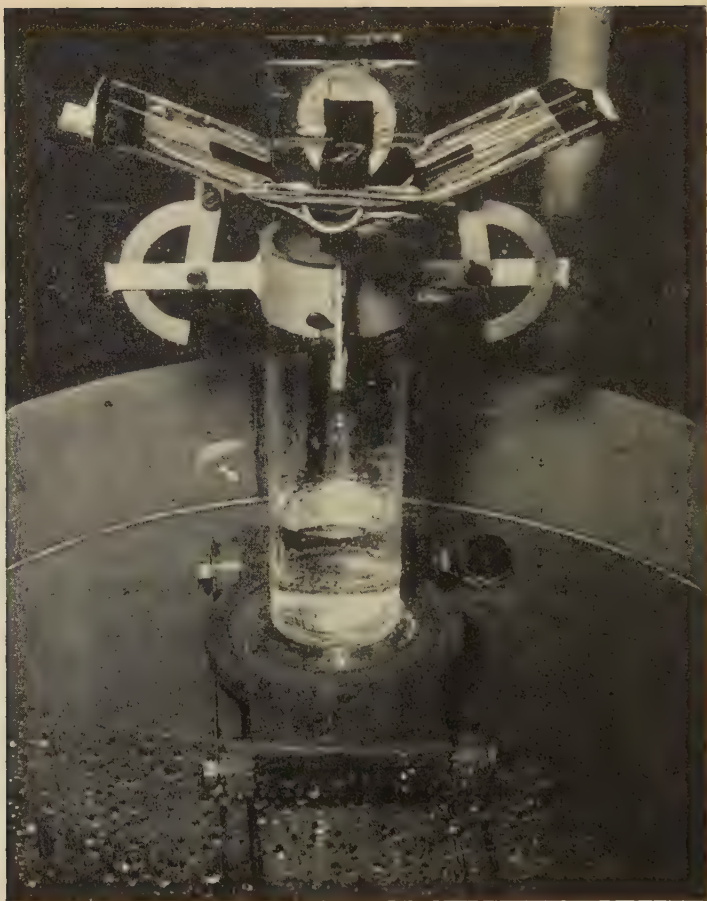


Fig. 24: Slides placed on the stand for exposure to rain.

after the paraffin rings and spore suspension drops of the EK 7/53 strain had been applied. The results are listed in Table 21, and also reproduced graphically in Fig. 25.

The increase in the tenacity as the particle sizes become smaller is clearly perceptible from Fig. 25. Ehlers (1953b) found that with an exposure to rain lasting 30 minutes, washing off takes place chiefly during the first minute of rainfall. Fig. 25 shows that it depends upon the particle size of the sulphur when the major part is washed off, and in fact how long the process lasts. Whereas for the fraction $2-6\ \mu$ there is no further loss of substance after 5 mm = 5 minutes of rain, this limit is not reached for the most effective fraction $< 2\ \mu$ until after 20 mm = 20 minutes of rain.

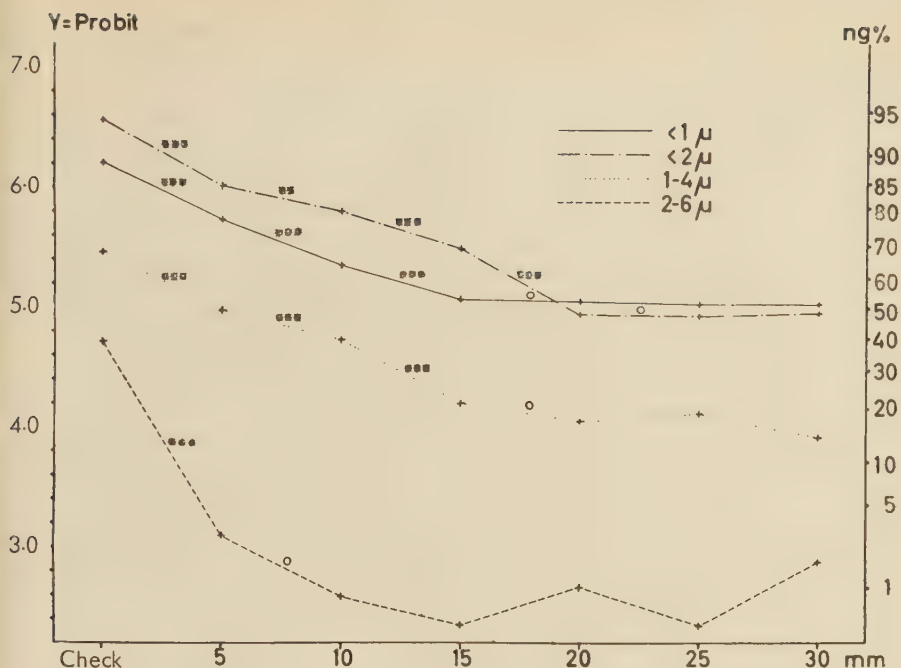


Fig. 25: Tenacity of wettable sulphur 80 of different particle sizes on slides.
24 hours' germination at $\sim 20^{\circ}\text{C}$. $N = 10$.

Table 21: Tenacity of wettable sulphur 80 of different particle sizes on slides.
Test carried out on conidia of the EK 7/53 strain.
24 hours' germination at $\sim 20^{\circ}\text{C}$. $N = 10$.

mm rain	2—6 μ			1—4 μ			< 2 μ			< 1 μ		
	$\bar{x}$ (ng %)	$\bar{x}$ (tr.)	$s_{\bar{x}}$	$\bar{x}$ (ng %)	$\bar{x}$ (tr.)	$s_{\bar{x}}$	$\bar{x}$ (ng %)	$\bar{x}$ (tr.)	$s_{\bar{x}}$	$\bar{x}$ (ng %)	$\bar{x}$ (tr.)	$s_{\bar{x}}$
check	43.0	41.0	0.89	70.6	57.2	0.79	94.6	76.9	1.05	89.6	71.3	0.85
5	11.0	19.2	0.83	53.4	47.0	1.11	85.6	68.0	1.34	78.8	62.7	0.83
10	9.2	17.5	0.72	44.4	41.8	0.99	80.6	64.0	0.83	66.8	54.9	1.04
15	8.8	17.1	0.81	28.0	31.9	0.91	71.4	57.8	0.98	56.6	48.8	0.67
20	9.4	17.8	0.67	24.2	29.4	0.97	52.0	46.2	0.98	56.0	48.5	0.66
25	8.8	17.2	0.63	25.8	30.5	1.04	51.6	45.9	0.95	55.2	48.0	0.73
30	10.0	18.2	0.98	21.2	27.1	1.80	52.8	46.6	0.97	55.4	48.1	0.69

$LSD_5 = 2.5$

$LSD_1 = 3.3$

$LSD_{0.1} = 4.3$

Values for 30 mm rain excluded, this being the only
way to keep the mean squares homogeneous.

Table 21a: Analysis of variance to Table 21, excluding the values for 30 mm rain.

	n	SQ	s ²	F
Total	239	78,892.68		
Exposure to rain (E. R.)	5	20,427.42	4,085.48	502.659***
Particle sizes (P.)	3	54,337.54	18,112.51	2,228.481***
E. R. × P.	15	2,372.13	158.14	19.457***
Remainder	216	1,755.59	8.13	

Accordingly, it is only the sulphur particles with a diameter of less than 2 μ that have an adequate tenacity on slides. With particles of up to 4 μ diameter, the efficacy of the deposit decreases greatly after exposure to rain. In view of the reasons mentioned above, no conclusions can be drawn from this finding with any degree of certainty as to the conditions on the leaf. Blumer and Kundert (1950) presumed, however, that the conditions on the leaf are somewhat more favourable, in other words that under certain circumstances particles of up to 4 μ diameter are also sufficiently resistant to rain.

F. Discussion

I was able to confirm on conidia of two strains of *Venturia inaequalis* the variations in the sensitivity of scab strains to fungitoxic substances (fungicides, fungistatica) observed by Palmiter (1934) on mycelium. This variable reaction of different strains may explain the frequently established fact that a preparation approved by the official plant protection service for the control of scab is not effective in many orchards. Apart from errors in application, the overwhelming occurrence of strains resistant to this particular preparation may be the cause of its failure. The occurrence of certain strains in one region may be encouraged by meteorological factors, in particular the wind direction (Herbst, 1938).

In this way, an explanation may also be furnished for the outstanding effectiveness of a combination of two different active substances in scab control tests. Loewel (1952) and Miller (1953) observed that the effect produced by a combined preparation was not equalled when the individual components were applied on their own. Sy (1954) reported that a synergistic effect was caused following the combined application of wettable sulphur and copper oxychloride against *Peronospora* on vines. The results I obtained in the spore-germination test permit the conclusion that two strains occurring simultaneously vary in their sensitivity to one or the other component of a combined preparation. By combining the two active substances, both strains can be controlled

satisfactorily. Naturally, this explanation of the synergistic effect given for *Venturia inaequalis* cannot be so easily applied to other fungi but requires reconsideration for each individual species.

With respect to the routine laboratory testing of the fungitoxic properties of chemical substances, the question is raised whether it is advisable to use single-spore cultures. There is the danger that substances will be rejected because they are not capable of killing the spores of one strain even though they may be effective against other strains. On the other hand, it is more or less technically impossible to test all substances which have to be examined regularly on several single-spore strains. Therefore, a separate decision must be reached in each particular case whether single or mass-spore cultures should be used. The use of single-spore cultures will then be given consideration when the results with conidia of mass-spore cultures vary too greatly.

My test results provide several suggestions and information also for the official testing of sulphur preparations. The Federal Biological Institute has reached agreement with the Pesticide Industry on certain standards for the testing of such preparations which "with respect to the active ingredient and the vehicles and auxiliaries, are very similar in quality and quantity". Preparations which comply with these standards in every respect will be approved without having to be subjected to a biological test (Trappmann, 1949). Definite methods have been worked out to check for consistency with standards (Zeumer, 1949; Zeumer and Fischer, 1951).

The wettable sulphur preparations are classified in three groups according to the provisional standards: colloidal sulphur liquid, colloidal sulphur solid, and wettable sulphur 80. For each group, a minimum sulphur content and a minimum suspension property have been laid down as requirements. According to more recent standards (Zeumer and Fischer 1951), provision is also made for the particle size to be tested in the case of colloidal sulphur. Due to the non-uniformity of the results obtained in fruit growing and viticulture, fixed standards have not yet been laid down for "wetable sulphur 80" preparations. The question is raised whether the three afore-mentioned standards provide suitable fundamentals for the evaluation of new wettable sulphur preparations, in other words whether "biological objects can be specified according to German industrial standardization" (Trappmann, 1949).

The chemical determination of the sulphur content is carried out in the manner described by Zeumer (1949) and by Zeumer and Fischer (1951), with a registration of the total sulphur. Sulphur forms which are more effective and less effective as fungicides, such as were found by De Ong (1924) and Feichtmeir (1949), as well as round and more adhesive, angular sulphur particles (cf. Windisch, 1901) cannot be separated from each other. Therefore, the biological effect cannot be determined together with the determination of the sulphur content.

The suspension property of wettable sulphur should primarily provide data on the particle size whereby a finding on the particle spectrum is not possible. In actual fact, however, the suspension property is not influenced solely by the particle size but may also be influenced by the addition of certain substances, in particular protective colloids. This test does not permit a statement to be made concerning the influence of such auxiliaries on the availability of the sulphur

on the leaf, in particular its tenacity and its redistribution on the newly grown foliage.

The particle size is determined microscopically. For colloidal sulphur a maximum diameter of $1\ \mu$ is quoted, with a minimum diameter of $1\ \mu$ for "wetttable sulphur 80" (Zeumer and Fischer, 1951). In the definition of the particle diameter, once again no allowance is made for the particle spectrum. My studies, however, have shown that the smaller particles within the particle spectrum are more effective than the larger ones. The proportional distribution of the individual particle sizes within the fixed limits has, therefore, a great influence on the efficacy of wetttable sulphur.

Accordingly, the specifications contained in the standards are not suitable for characterizing the biological effect of wetttable sulphur preparations and the possibility of their technical application. The approval of wetttable sulphur and its classification in one of the groups provided for in the list of pesticides, made on this basis, must therefore, inevitably lead to the individual wetttable sulphur preparations being heavily overrated or underrated. As in the past, it is, therefore, still impossible to do without a biological test.

According to my investigations, no differences of any importance are to be anticipated between the effect of wetttable sulphur against conidia of *Venturia inaequalis* on slides and its effect against the same on the leaf. However, in order to clarify the question once and for all as to whether the testing of the efficacy of wetttable sulphur in the laboratory on inert material is sufficient for making an evaluation of wetttable sulphur, closer investigations will have to be carried out on such material and on the green plant under controlled conditions and in the field under natural conditions.

G. Summary

1. A condition for carrying out the spore-germination test on slides is the ability to make a satisfactory application of the fungicide. A pendulum sprayer has been developed for applying fungicides to slides. This sprayer permits an exact dosing of the applied quantity.
2. Vigorous sporulation and a high and uniform percentage germination of the conidia of the test fungus are further prerequisites for the slide-germination test. A regular change of the nutrient medium maintains the sporulation of *Venturia inaequalis* and increases the power of the conidia to germinate. Seven day old cultures mostly contain an adequate number of conidia with a high germinating capacity. Temperatures of approximately 18 to 22°C should be maintained during cultivation and while the germination test is in progress. No allowance need be made for light and pH values.
3. Eight single-conidial cultures were classified in two different types, according to colour and growth of mycelium, conidial form and germination, length of germ tubes and development of appressoria.

The conidia of the two scab strains EK 4/53 and EK 7/53 differed in their sensitivity to fungitoxic substances, with variations in the reaction to copper oxychloride, TMTD, zinc dimethyl dithiocarbamate and wetttable sulphur.

The two strains EK 4/53 and EK 7/53 had not lost their ability to cause infection after 2½ years of cultivation on artificial nutrient media. The resistance of conidia of both strains to wettable sulphur was increased by means of a leaf passage, although the basic variations in their sensitivity remained the same.

4. The effectiveness of wettable sulphur increases as the size of the particles decreases. A reduction of the particle size to less than 1 μ is not accompanied by a further increase in effectiveness.

The effectiveness of wettable sulphur increases as the temperature rises between 5° C and 25° C.

In addition to being fungistatic, wettable sulphur also has fungicidal properties. The initial fungicidal effect of wettable sulphur, like the fungistatic effect, depends largely upon the temperature.

A method has been described for testing the persistence of fungicides in the laboratory.

The persistence of wettable sulphur decreases as the particle size becomes smaller. The drop in the effectiveness of wettable sulphur increases in proportion to increases in temperature, relative humidity and the time wettable sulphur is exposed to light. The light factor had no considerable influence on persistence under the conditions prevailing in the test.

The tenacity of wettable sulphur deposits was tested in the laboratory by means of a newly developed apparatus. Wettable sulphur with particles of less than 2 μ diameter is sufficiently resistant to rain on slides. Particles with a diameter exceeding 4 μ do not adhere well. It depends upon the particle size of wettable sulphur when the majority of the sulphur will be washed off, and in fact how long this process will last.

Taking into consideration efficacy, persistence, tenacity and the danger of burns on tall plants, the most favourable particle size of a wettable sulphur used for scab control is 1—4 μ diameter with a maximum at 1—2 μ diameter.

5. An approval of wettable sulphur preparations according to standards covering physical and chemical properties only, will not do justice to the biological efficacy of the preparations. It is essential also to carry out a biological test on suitable test objects to give full satisfaction on this point.

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